The effects of ageing method and time on meat quality attributes of Springbok (*Antidorcas marsupialis*) *Longissimus thoracis et lumborum* (LTL) muscle

by

DAPHNE ENID WABULE

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Department of Food Science, Faculty of AgriSciences, Stellenbosch University

Supervisor: Prof. Louwrens Hoffman
Co-supervisor: Dr. Jeannine Marais
Co-supervisor: Prof. Pieter Gouws

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Declaration

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Date: 02/05/2020

Summary

The purpose of this study was to determine the effect of ageing on springbok (*Antidorcas marsupialis*) meat quality. Ageing methods [skin-on and vacuum pack ageing (VAC)] and time (6-9 days) previously recommended for ageing springbok were applied to *Longissimus thoracis et lumborum* (LTL) muscles from twenty-four springbok harvested from Witsand, Western Cape. Carcass and muscle characteristics, physical attributes [ultimate pH (pH_u), colour, weep loss, cooking loss and Warner-Bratzler shear force (WBSF)], proximate composition, microbiological characteristics, fatty acid profiles, volatile compound profiles and descriptive sensory profiles were thereafter analysed.

Dressing percentage was lower ($p = 0.047$) in skin-on aged carcasses (54.5 ± 2.90%) than carcasses from which LTL muscles were extracted for VAC ageing (56.8 ± 4.81%). Additionally, there was a loss of 5.17 ± 1.28% weight from skin-on carcasses possibly because of evaporation from skin and exposed muscle surfaces during ageing. No *Escherichia coli* (*E. coli*) was detected in the meat samples. The latter shows that not only was it possible to skin the aged carcasses without compromising the microbiological safety of meat, but that skin-on ageing did not necessarily present greater risk of contamination. The aerobic plate counts (APC) of aged meat across all treatments (1.7 to 2.5 log CFU/g) were well below the recommended lower limit (APC = 3.5 log CFU/cm^2) safe for human consumption.

Female springbok had higher ($p = 0.004$) intramuscular fat (IMF) content than males while male springbok had higher ($p = 0.016$) moisture content than females. There were no effects ($p > 0.05$) of ageing method or ageing time on the proximate composition of aged springbok. The pH_u increased ($p = 0.013$) slightly with ageing time.

An interaction between ageing method and time was observed for some of the bloomed muscle colour ordinates, specifically, a* and chroma values that followed similar trends ($p = 0.050$ and 0.035, respectively). The b* and hue angle values were affected by ageing time ($p = 0.028$ and 0.026 respectively) with day 6 having the lowest recorded b* and hue angle values. The Warner-Bratzler Shear Force (WBSF) values of the muscles for all treatments were generally low (29.26 ± 11.16 N) with exception of two samples that were uncharacteristically tough (64.17 ± 17.86 N); likely as a result of ante-mortem stress during harvesting.

Numerous differences in the fatty acid profile linked to sex were observed and likely occurred as result of the effect of fat content (IMF) on fatty acid profile of meat. The higher IMF content of female springbok resulted in lower total PUFA content ($p = 0.005$) in females than males. Despite the increased likelihood of oxidation during ageing due to the abundance of unsaturated fatty acids in springbok meat, the polyunsaturated fatty acid to saturated fatty acid (PUFA:SFA) ratios and omega-3 to omega-6 ratios (n-3:n-6 PUFA) across all treatments were within the ranges recommended for a healthy diet (mean PUFA:SFA = 0.65 ± 0.42, mean n-6:n-3 PUFA =0.58 ± 0.15).

The occurrence of various volatile compounds typically linked to lipid oxidation in the aged springbok meat suggested that lipid oxidation played a key role in aroma compound formation during this study. Additionally, the higher number of volatile compounds (53 compounds) observed in aged springbok meat during this study indicated volatile compounds increased during the process of ageing. However, no strong correlations were calculated between volatile compounds and aroma attribute scores from Descriptive Sensory Analysis (DSA) making it difficult to identify the roles played by specific volatile compounds in aroma and flavour perception.

With regard to ageing as a way of improving the tenderness of meat, the tenderness DSA scores were high (> 65) across all treatments showing that all ageing treatments applied in this study were capable of producing meat with suitable tenderness. There were notable effects of ageing method, time and sex on the sensory profile of the aged springbok meat; most notably the interaction between ageing method and time for gamey aroma ($p = 0.001$) and texture attributes ($p < 0.0001$). Additionally, lower DSA scores (< 10) for negative attributes such as residue and liver-like attributes highlights the function of ageing as a way to improve meat texture and flavour. However, as most reported differences in the sensory scores were below 10 on a 100-point scale, it is unclear if consumers would be able to notice them.

Opsomming

Die doel van hierdie studie was om die effek van veroudering op springbok (*Antidorcas marsupialis*) vleiskwaliteit te bepaal. Die verouderingsmetodes [vel-aan en vakuumverpakking veroudering] en verouderingstyd (6-9 dae) wat voorheen aanbeveel is vir die veroudering van springbok was toegepas op die *Longissimus thoracis et lumborum* (LTL) spiere van vier-en-twintig springbokke vanaf Witsand, Wes-Kaap geoes. Gevolglik was die karkas en spiere se eienskappe, die fisiese eienskappe [finale pH (pH_u), kleur, uitvloei vogverlies, kookverlies en Warner-Bratzler skeurkrag (WBSF)], proksimale samestelling, mikrobiologiese eienskappe, vetsuurprofiel, vlugtige komponente profiel en die beskrywende sensoriese analise profiel geanaliseer.

Die uitslagpersentasie van die vel-aan verouderde karkasse (54.5 ± 2.90%) was laer ($p = 0.047$) as die karkasse waarvan die LTL spiere vakuumverouderd (56.8 ± 4.81%) was. Daarbenewens was daar 'n verdampingsverlies van 5.17 ± 1.28% gewig vanaf die vel-aan karkasse wat moontlik as gevolg van verdamping vanaf die vel en die blootgestelde spiere gedurende veroudering was. Geen *Escherichia coli* (*E. coli*) was opgespoor in die vleismonsters nie. Laasgenoemde wys dat dit moontlik is om vel-aan karkasse af te slag sonder om die mikrobiologiese veiligheid van die vleis prys te gee (selfs na vel-aan veroudering), maar dit wys ook dat vel-aan veroudering nie noodwendig 'n groter risiko vir kontaminasie is nie. Die aërobiese plaattellings van die verouderde vleis van alle behandelings (1.7 to 2.5 log CFU/g) was ver onder die laer limiete (3.5 log CFU/cm^2) wat vir menslike verbruik aanbeveel word.

Vroulike springbokke het hoër ($p = 0.004$) intramuskulêre vetinhoud gehad as manlike diere, waar manlike springbokke hoër ($p = 0.016$) voginhoud gehad het as vroulike diere. Verouderingsmetode en tyd het geen effek ($p > 0.05$) op die proksimale samestelling van die verouderde springbokke gehad nie. Die pH_u het toegeneem ($p = 0.013$) met verouderingstyd en was gevolglik die laagste op dag 7.

Verouderingsmetode en tyd het 'n interaksie gehad vir die a* en chroma waardes wat gevolglik soortgelyke tendense gevolg het ($p = 0.050$ and 0.035, onderskeidelik). Die b* en kleur hoek waardes was beïnvloed deur verouderingstyd ($p = 0.028$ and 0.026, onderskeidelik) met dag 6 wat die laagste b* en kleur hoek waardes getoon het. Die Warner-Bratzler skeurkrag waardes vir alle behandelings was oor die algemeen laag (29.26 ± 11.16 N) met die uitsondering van twee monsters wat onverwags taai was (64.17 ± 17.86 N), waarskynlik as gevolg van ante-mortem spanning gedurende die oes van die diere.

Daar was talle verskille in die vetsuurprofiele wat toegeskryf was aan verskille tussen geslagte as 'n resultaat van die effek wat geslag op die vetinhoud en gevolglik op die vetsuurprofiel van vleis het. Die hoër intramuskulêre vetinhoud van vroulike springbokke het gelei tot 'n laer poli-onversadigde vetsuurinhoud ($p = 0.005$) in vroulike diere in vergelyking met manlike diere. Ongeag die moontlikheid dat oksidasie tydens veroudering gewoonlik toeneem as gevolg van die oorvloed van onversadigde vetsure in springbokvleis, was die poli-onversadigde tot versadigde vetsuurverhouding en die omega-3 tot omega-6 poli-onversadigde vetsuurverhouding vir alle

behandelings binne die reeks wat vir gesonde menslike diëte voorgestel word (gemiddelde van 0.65 ± 0.42 en 0.58 ± 0.15, onderskeidelik).

Die teenwoordigheid van ʼn verskeidenheid vlugtige komponente in verouderde springbok vleis wat tipies aan lipied-oksidasie toegeskryf word, stel voor dat lipied-oksidasie ʼn belangrike rol gespeel het in die aroma komponente wat gevorm is gedurende veroudering in hierdie studie. Die hoër getal vlugtige komponente (53 komponente) wat in verouderde springbokvleis in hierdie studie opgelet is, wys dat die totale aantal vlugtige komponente toegeneem het met die proses van veroudering. Daar was egter geen sterk korrelasies tussen die vlugtige komponente en die aroma eienskappe van die beskrywende sensoriese analise nie. Laasgenoemde maak dit moeilik om die rolle van spesifieke vlugtige komponente in die aroma en geurpersepsie van springbokvleis te identifiseer.

Met betrekking tot veroudering as ʼn manier om die sagtheid van vleis te verbeter, was die sagtheid tellings van beskrywende sensoriese analise hoog (> 65) vir alle behandelings wat toon dat alle verouderingsbehandelings wat in hierdie studie toegepas is, gelei het tot die produksie van vleis met gepaste sagtheid. Verouderingsmetode, verouderingstyd en geslag het noemenswaardige invloede op die sensoriese profiel van verouderde springbokvleis gehad; mees opmerklik was die verskil in wildsvleis aroma ($p = 0.001$) en tekstuureienskappe ($p < 0.0001$) met ʼn interaksie tussen verouderingsmetode en tyd. Daarbenewens beklemtoon die laer beskrywende sensoriese analise tellings (< 10) vir negatiewe eienskappe soos residu en leweragtige eienskappe die funksie van veroudering om vleis se tekstuur en geur te bevorder. Aangesien die meeste gerapporteerde verskille in die sensoriese tellings op ʼn 100-punt skaal laer as 10 was, is dit onduidelik of verbruikers dit sou kan opmerk.

This thesis is dedicated to each individual I have run this race with.

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NOTES

This thesis is presented in the format prescribed by the Department of Food Science, Stellenbosch University. The language, style and referencing used are as per the *International Journal of Food Science and Technology*. This thesis is a compilation of individual chapters and some degree of repetition is inevitable.

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CHAPTER 1
General introduction

1.1 Background and research aim

The value of the game meat industry in South Africa has been steadily increasing since the early 1970s. There has been a sharp rise in production for the past two decades with peak production of 46,000 t of game meat recoded in 2011 (Fig. 1.1) (FAOSTAT, 2017). Increasing health and environmental consciousness among consumers as well as the availability of game meat appear to have spurred an increase in consumption of game meat in South Africa (Wassenaar *et al.*, 2019).

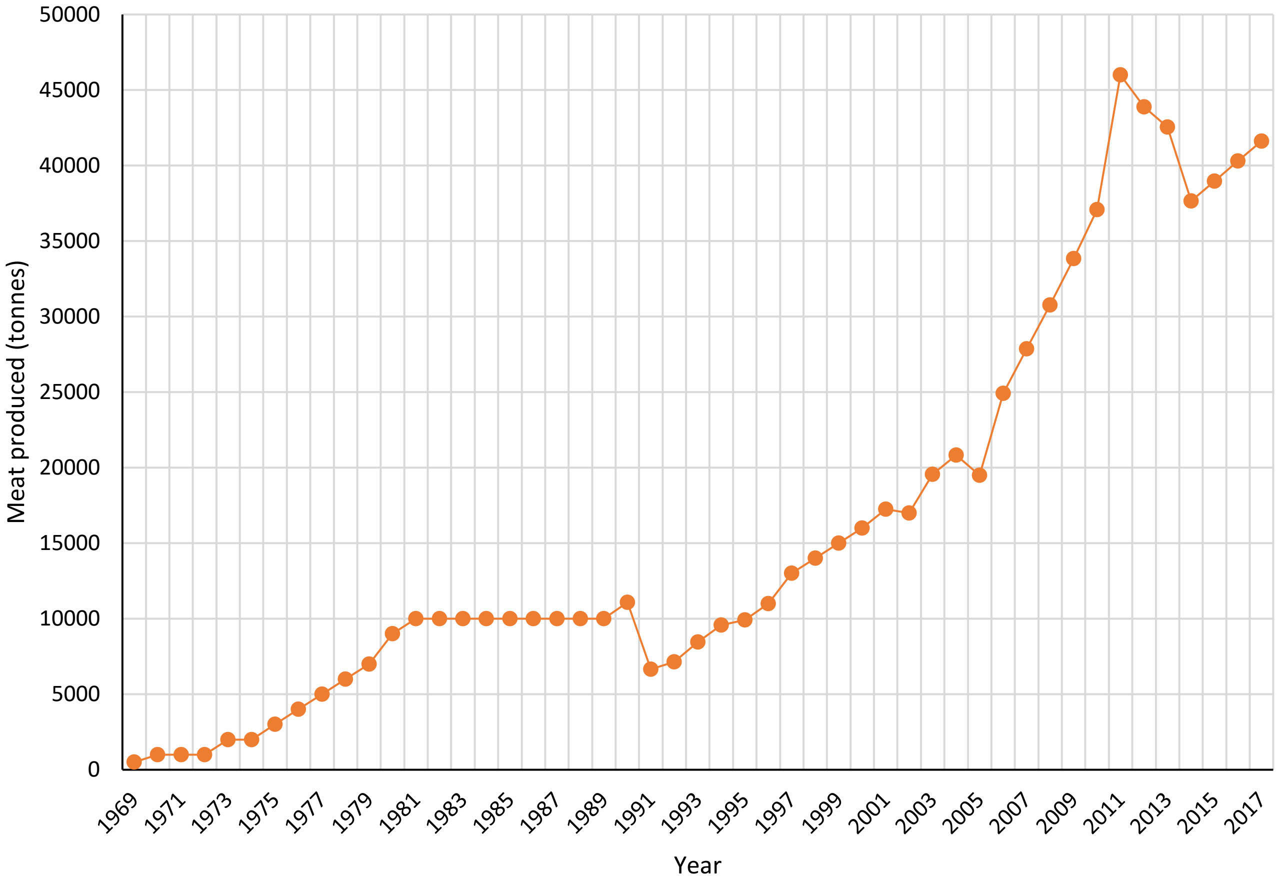

Figure 1.1 The trend in game meat production in South Africa from 1970 to 2017 (FAOSTAT, 2017).

Springbok (*Antidorcas marsupialis*) are indigenous to southern Africa and therefore better adapted to adverse climatic conditions, such as droughts, than domesticated meat species like cattle and sheep (Skinner, 1996). There has been a noted decline in herd numbers of cattle and sheep in recent years following droughts (Anonymous, 2017) in South Africa (Fig. 1.2). The free ranging and extensive nature of game ranching in South Africa also appeals to a growing base of environmentally conscious meat consumers (Hoffman, 2007; Wassenaar *et al.*, 2019). Finally, amidst growing concerns of the health implications of consuming red meat (Gehring, 2017; Wood, 2017), springbok meat has been shown to be a low fat, high protein and nutrient dense alternative red meat (Hoffman, 2007; Hoffman *et al.*, 2007a,b; North & Hoffman, 2015). Yet with the increased demand for game meat comes the need to evaluate and standardise production across the industry in order to ensure consistent meat quality (Hoffman *et al.*, 2004).

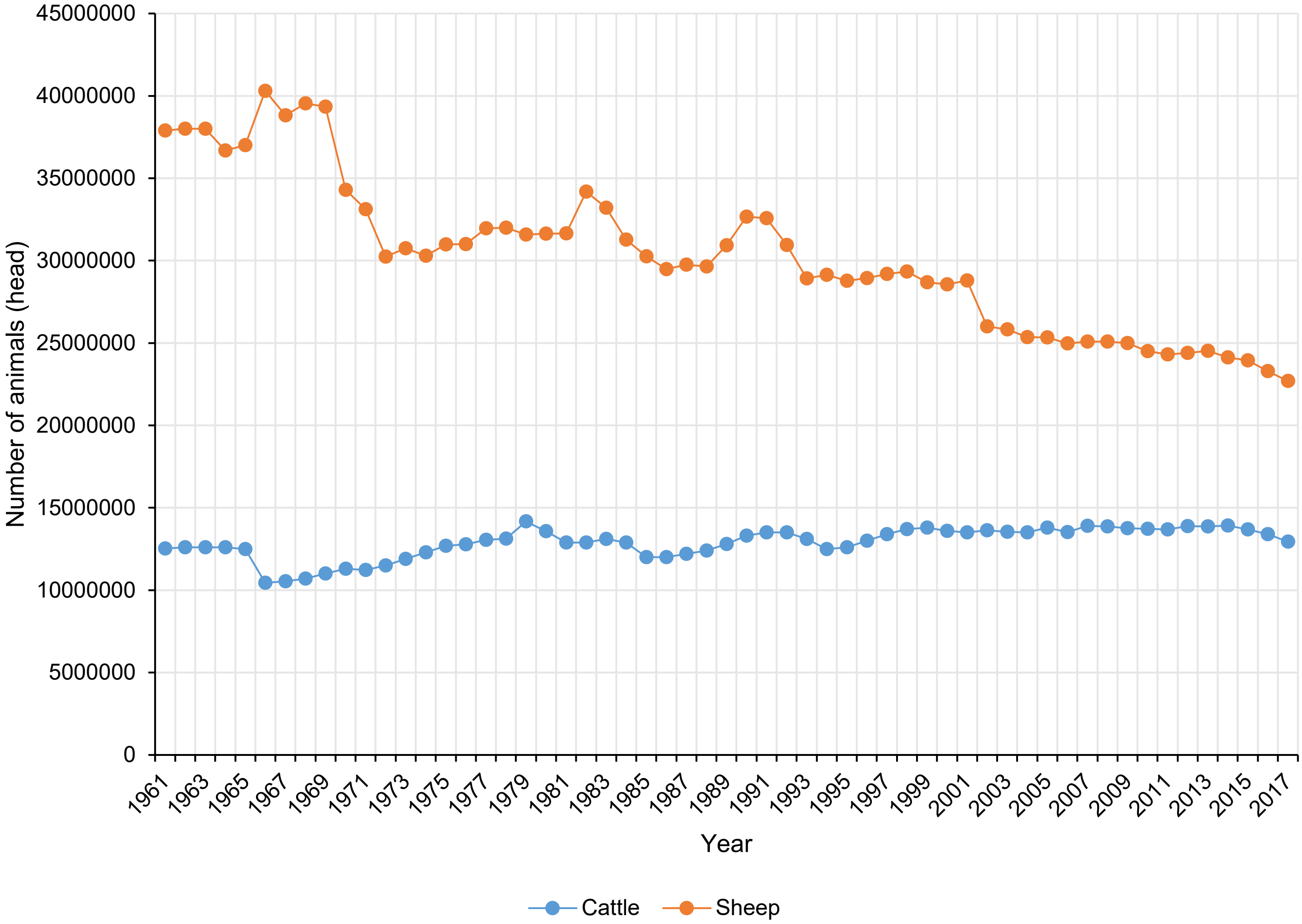

Figure 1.2 The change in herd numbers of cattle and sheep in South Africa from 1961 to 2017 (FAOSTAT, 2017).

The free market nature of the South African game ranching industry allows for the sale of meat sourced by both large scale commercial farmers as well as small scale producers (Hoffman *et al.*, 2004; Hoffman, 2007). This has resulted in different carcass processing methods being applied depending on the scale of harvesting. Typically, large scale harvesting operations span several days thus requiring carcasses to be stored skin-on in a chiller for several days before further processing (Van Schalkwyk & Hoffman, 2016). Skin-on ageing has previously been recommended for springbok meat (Jansen van Rensburg, 1997) although space restrictions and the possibility of cross-contamination from skins (Bell, 1997) pose some disadvantages for this ageing method.

Ageing in vacuum packaging has more recently been recommended for flavour improvement of springbok meat (North & Hoffman, 2015). Not only does this method allow for the selective ageing of the more expensive prime cuts but it also frees up chiller space allowing for faster turnover times. However, on large scale operations, skin-on ageing allows for the reduction of transport and labour costs in instances where the processing plant is a long way from the harvesting ground.

Ageing is a technique that has long been applied to improve tenderness and flavour of meat (Lawrie & Ledward, 2006). When considering ageing springbok meat, the major benefit is in flavour improvement as unaged springbok meat has been shown to be tender (North & Hoffman, 2015). Additionally, the beneficial fatty acid profile of springbok meat must be kept in consideration as the possibility for oxidation during ageing exists (Wood *et al.*, 2003). Finally, the microbial quality of meat and therefore safety should not be compromised in the process of ageing.

Ageing has previously been shown to affect attributes of game meat quality with the method used and the duration of the process impacting quality (North & Hoffman, 2015; Maggiolino *et al.*, 2018). In order to mitigate inconsistencies in springbok meat quality, it is therefore important to understand the impact of ageing methods applied on the meat quality. Previous studies on aged springbok meat reported improved tenderness and flavour (Jansen van Rensburg, 1997; North & Hoffman, 2015) and increased microbial counts (Buys *et al.*, 1997). From these studies, skin-on ageing for up to 10 days was recommended for springbok meat (Jansen van Rensburg, 1997) whilst ageing in vacuum packaging was recommended for a maximum of 8 days post mortem (North & Hoffman, 2015).

The aim of this study was therefore to assess the ageing times and methods recommended for springbok meat (Jansen van Rensburg, 1997; North & Hoffman, 2015). The differences in carcass characteristics (dressing percentage and weight loss during ageing), physical attributes (ultimate pH, colour, weep loss and cooking loss), microbial quality, proximate composition, fatty acid profile, volatile compound profile and ultimately the sensory profile as a result of ageing methods and time applied were therefore assessed.

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CHAPTER 2

A review of the effects of ageing method and time on meat quality attributes

2.1 Introduction

The game meat industry in South Africa has come far since its early days and the growth seen in the industry thus far signals the potential of game meat products in South Africa. The growth of the game meat industry and increased understanding of game meat quality means that a critical understanding of carcass handling practices, and how they affect meat quality, is necessary to provide meat with consistently high quality.

The effects of ageing on meat, particularly beef, have been extensively studied for many decades; however, notably less research has been conducted on game meat (Jansen van Rensburg, 1997; North & Hoffman, 2015; Needham *et al.*, 2020). Although a certain amount of extrapolation from research on beef can be done regarding the effects of ageing on game meat, critical differences between the two such as greater unsaturated fatty acid content and myoglobin content as well as the harvesting procedure for game indicate that there may be changes during ageing that are unique to game meat. For example, higher proteolytic activity in game meat species compared to beef (Barnier *et al.*, 1999; Farouk *et al.*, 2007) means that game meat species such as springbok has a shorter optimal ageing period of about six to ten days (Jansen van Rensburg, 1997; North & Hoffman, 2015) than beef. However, there is little research on the effects of ageing on meat from South African game species (Jansen van Rensburg, 1997; North & Hoffman, 2015; Needham *et al.*, 2020). Therefore, understanding how ageing affects game meat quality attributes will be key in producing game meat with consistent quality.

2.2 The game meat industry in South Africa

The game industry in South Africa is a free market enterprise that allows individual game ranchers and meat producers to operate (Hoffman *et al.*, 2004). Furthermore, Carruthers (2010) reported a shifting focus of consumers from aesthetic and ideological uses for game species to a utilitarian view focused on biltong and meat production. Although one of the major income earners for the game industry so far has been live sales of animals, decreasing prices offered for these animals warrants an alternative use for game species (Hoffman *et al.*, 2003; Hoffman, 2007). Sales of game meat have the potential not only to provide additional income to farmers but also to play a role in improving food security (North *et al.*, 2016; Taylor *et al.*, 2016). Although hunting game for biltong production has been a major earner for the South African game ranching industry (Hoffman, 2007; Van der Merwe *et al.*, 2014; Taylor *et al.*, 2016), there is potential for meat production from game species to contribute to the red meat supply in South Africa (North & Hoffman, 2015; Taylor *et al.*, 2016).

The game meat industry is growing in South Africa and overseas. An increase in game meat production has been observed in South Africa over the past two decades with production growing from below 20,000 tonnes of game meat at the turn of the century to approximately 42,000 tonnes in 2017 (FAOSTAT, 2017a). In 2017, a survey of member countries of the United Nations Economic Commission for Europe (UNECE) indicated that total exports of game meat from the region were estimated at 133,000 tonnes per year (valued at €340 million) and imports of approximately 270,000 tonnes per year valued at €270 million (FAOSTAT, 2017b; UNECE/FAO, 2018). Soriano *et al.* (2016) also noted the gradual increase in demand for game meat in Europe especially in developed countries.

Factors surrounding the production, harvesting and the very nature of the game meat make it an ideal product for today's environmentally concerned and health conscious consumers (Hoffman & Wiklund, 2006). Game meat could potentially be described as an organic product due to the extensive nature of most ranches in South Africa, as well as the lack of pesticide and fertiliser use during the production process (Hoffman, 2007; D'Amato *et al.*, 2013; Wassenaar, 2016). Consumers also associate game meat with nature and perceive it as a healthy and novel product (Hoffman *et al.*, 2003; Radder & Grunert, 2009; Wassenaar *et al.*, 2019). Therefore, the growing trend towards increased game meat consumption should be fostered in South Africa.

Radder and Grunert (2009) reported that many South Africans consumed red meat more than three times a week, however, game meat was not regularly consumed more than twice a week. Furthermore, Carruthers (2010) stated that it was unlikely that venison/game meat would ever be able to fulfil its promise to replace conventional meats in the diet of red meat consumers. However, the increasing lack of suitable grazing area in the arid regions of South Africa has led to a stagnation in cattle and decline in sheep herd numbers and increased red meat imports (Thomas, 2012). As game animals are better adapted to these arid conditions, game meat production could be a more feasible alternative than increasing South Africa's red meat imports (Thomas, 2012). However, there are also negative perceptions by consumers towards game meat that need to be overcome in order to increase mainstream consumption of game meat (Wassenaar *et al.*, 2019). Consumers have been noted to perceive game meat as dry (Radder & Grunert, 2009), a seasonal product (Hoffman *et al.*, 2004; Hoffman & Wiklund, 2006; Hoffman, 2007) and of inconsistent quality (Hoffman *et al.*, 2004). Inconsistency in game meat quality is as a result of variation of several factors that affect meat quality including harvesting method, slaughter handling techniques (Hoffman & Wiklund, 2006) as well as meat processing techniques (North *et al.*, 2016).

2.3 Springbok

Among the various game species present in South Africa, springbok (*Antidorcas marsupialis*) is the most cropped, sold, consumed and exported species (Hoffman *et al.*, 2003, 2004, 2007a; Hoffman, 2007; Thomas, 2012). Within a herd, springbok show an annual growth rate of 25 – 35% with populations in Southern Africa estimated at 2 to 2.5 million animals as of 2013 (Thomas, 2012; Skinner, 2013). Their large and growing population coupled with the adaptation of springbok to the southern African climate make it the ideal species for game meat production.

As early as the 1980s, the dominance of springbok in the game meat industry was visible with springbok making up 75% of game exports from South Africa (Hoffman, 2007). In 2012, it was reported that South Africa was earning ZAR 60 to 70 million annually from the export of springbok meat. Unfortunately, the export of game meat from South Africa to the European Union market is currently prohibited due to the poor management of foot and mouth disease zones (Uys, 2015); Namibia however is still able to export springbok meat to the European Union (Van Schalkwyk & Hoffman, 2016).

2.3.1 Springbok meat

2.3.1.1 The animal

The springbok is an African antelope that is widely distributed across South Africa. Springbok are social animals that live in herds. Their natural habitat is the arid and semi-arid plains of Africa. They are able to survive the unpredictable harsh climatic conditions and poor nutritional conditions through a variety of adaptations. Springbok are able to survive the poor nutritional conditions by being mixed feeders as they are able to feed from both shrubs (browse) and grass (graze) thereby making use of a wider range of natural vegetation than other domesticated species (such as sheep) in the same area (Skinner, 1996). Additionally, when forage is abundant, springbok increase their intake two-threefold. Springbok also minimise water requirements by morphologically being able to reduce overheating as well as reabsorbing water from excrement in order to reduce water loss. Finally, springbok are able to breed throughout the year unlike other game species that have restricted breeding seasons. They reproduce rapidly when conditions are favourable thus allowing their population to grow constantly (Skinner, 1996).

The widespread distribution of springbok in their natural habitat has earned them "Least Concern" status on the Regional Red List in 2016 as well as on the Global Red List in 2008 (Anderson *et al.*, 2016). Their population in South Africa is estimated to have grown 8-23% between 1994 and 2015. However, there were regional declines reported in populations within South Africa, likely because of environmental stresses, predation and degradation from livestock overgrazing (Anderson *et al.*, 2016). Skinner (1996) recommended an annual cropping rate of 30-40% depending on the season with the higher rate advised in years of good rainfall and two lambing seasons.

2.3.1.2 Composition

Springbok reach 80% of their ultimate mass quite quickly with male springbok taking 40 weeks to achieve this weight and females 28 weeks (Conroy, 2005). This rapid growth rate makes springbok ideal for meat production. Domesticated meat species reach slaughter age at about the same time or even later when compared to springbok, such as cattle (less than thirty-six months), pigs (five to ten months) or sheep and goats (six to twelve months (FAO, 1991a). Springbok have a dressing percentage of approximately 56% by 12 weeks and carcasses are generally composed of approximately 83% lean, 13% bone and 4% fat (Skinner, 1996). Increase in percentage lean and fat with age follow a sigmoidal pattern with growth starting to slow down after 28 weeks (Skinner, 1996).

The proximate composition of springbok meat as reported in previous studies is shown in Table 2.1. Sex and production region are two factors that have been documented to affect the proximate composition of springbok meat. In relation to sex, there have been some significant differences reported in the chemical composition between female and male springbok (Hoffman *et al.*, 2007b; Neethling *et al.*, 2018). Higher intramuscular fat (IMF) and lower moisture content has been reported in female springbok than in male springbok (Hoffman *et al.*, 2007b; Neethling *et al.*, 2018). Additionally, an inverse relationship between moisture content and IMF has been observed in springbok meat ($r = -0.817$) (Neethling, 2016a), as well as in meat from other species (Legako *et al.*, 2015). Springbok meat is considered lean due to its low IMF content of less than 3% IMF (Table 2.1). Hoffman *et al.* (2007b) also found significant effects of age on proximate composition. Sub adults and adults had higher IMF content than lambs. Moisture and IMF content of springbok meat were also affected by the production region (Hoffman *et al.*, 2007b; Neethling *et al.*, 2018). These could be as a result of variations in vegetation in the different production areas in South Africa thus affecting the quantity and quality of nutrients available for the animals (Hoffman *et al.*, 2007b). It was also noted from the same study, that the proximate composition of springbok meat was similar to that of farmed deer.

Table 2.1 The mean chemical proximate composition (g/100 g) of springbok meat as reported in previous studies

Moisture	Protein	Fat	Ash	Reference
74.7	-	1.7	-	Von La Chevallerie, 1972[1]
72.16	24.18	2.27	1.32	Du Buisson, 2006[1]
72-74	22.90-24.20	1.34-3.46	1.24-1.37	Hoffman *et al.*, 2007b[2]
73.8 ± 0.33	22.1 ± 0.20	3.1 ± 0.24	1.1 ± 0.03	Neethling *et al.*, 2018[3]

1 Mean proximate composition reported

2 Range of proximate composition reported

3 Mean ± standard error proximate composition reported

Springbok meat generally has a higher unsaturated fatty acid content as compared to the meat from other domesticated species (Table 2.2) which is attributed to the free-range nature of game farming as the animals graze and browse on the existing vegetation (Wood *et al.*, 2003; Hoffman & Wiklund, 2006). Stearic acid (24 – 27.02% of total fatty acid content) is the main saturated fatty acid (SFA) found in springbok meat. Oleic acid (16 – 20% of total fatty acid content) is the main monounsaturated fatty acid (MUFA) present and α-linolenic acid the main polyunsaturated fatty acid (PUFA) present in springbok meat (Hoffman *et al.*, 2007c). The cholesterol content of springbok meat ranges from 54 to 59 mg/100g of meat (Hoffman *et al.*, 2007c) and mean PUFA to SFA ratios (PUFA:SFA) ranging from 0.96 to 1.18 (Hoffman *et al.*, 2007c) as well as 0.13 to 1.55 (Neethling *et al.*, 2018) have been reported. The PUFA:SFA ratios are generally well above the minimum of 0.4 recommended for a healthy diet (Schmid, 2011).

The influence of location on the fatty acid profile of springbok meat is attributed to differences in natural vegetation in production areas (Neethling *et al.*, 2018) while the influence of sex on fatty acid profile is attributed to differences in IMF content between male and female springbok (Clausen *et al.*, 2009). Thus, studies on springbok meat fatty acid profile present some contradictory findings. For example, while Hoffman *et al.* (2007c) found minor effects of production region on the fatty acid profile of springbok meat from similar biomes, Neethling *et al.* (2018) found more effects when animals were harvested from different biomes as well as significant interactions ($p \leq 0.05$) between sex and production region. Generally, female springbok had higher oleic acid content ($g.kg^{-1}$ of muscle and percentage composition) than male springbok regardless of production region (Hoffman *et al.*, 2007c; Neethling *et al.*, 2018). While Hoffman *et al.* (2007c) found higher ($p < 0.05$) percentage contribution of MUFA to the overall fatty acid profile of male springbok than female, Neethling *et al.* (2018) found that MUFA content across production regions was higher ($p < 0.001$) in female springbok than males.

Table 2.2 The mean (mg/g) of fatty acids present in the intramuscular fat of meat derived from different species

Fatty acid	**Springbok**[a]	**Beef**[b]	**Lamb**[b]	**Pork**[b]
Stearic acid	6.85	5.07	8.98	2.78
Oleic acid	6.21	13.95	16.25	7.59
Linoleic acid	4.87	0.89	1.25	3.02
γ linolenic acid	0.02	nd*	nd	0.01
α linolenic acid	1.49	0.26	0.66	0.21
Eicosadienoic acid	0.04	nd	nd	0.09
Arachidonic acid	1.41	0.22	0.29	0.46
Eicosapentaenoic acid	0.52	0.10	0.21	0.65

*nd not detected

[a] Neethling *et al.* (2018)

[b] Enser *et al.* (1996)

Overall, springbok meat is a nutrient dense and low fat red meat (Hoffman *et al.*, 2007b,c) that is derived from a species that is suitably adapted to the climate and natural vegetation in South Africa (Skinner, 1996). However, maintaining consistent meat quality and supply on the market (Hoffman *et al.*, 2004; Wassenaar *et al.*, 2019) will be vital in fostering widespread adoption of springbok and game meat at large in the South African diet.

2.3.1.3 Factors influencing meat quality

Physical, chemical, sensory and microbiological characteristics are the major aspects influencing meat quality. Factors that affect these characteristics therefore play an important role in determining final meat quality.

During the conversion of muscle to meat, post-mortem glycolysis results in a build-up of hydrogen ions, which causes a drop in pH over time. Under normal conditions, muscle pH will drop from 7.2 to an ultimate pH (pH_u) of ~ 5.6 in approximately 24h (North *et al.*, 2016; Matarneh *et al.*, 2017). The extent of post-mortem glycolysis and the subsequent pH decline is dependent on both glycogen content of muscles ante-mortem and the activity of glycolytic enzymes post-mortem (Matarneh *et al.*, 2017). Meat with a high pH_u can be classified as dark, firm and dry (DFD) and will generally have a shorter shelf-life (Wiklund *et al.*, 1995; Matarneh *et al.*, 2017; Shange *et al.*, 2019). Ante-mortem stress can cause rapid glycogen depletion resulting in insufficient post-mortem glycolysis thereby producing meat with pH_u values typically greater than 6 (Wiklund *et al.*, 1995; Matarneh *et al.*, 2017). The predisposition of game to the effects of stress (Hoffman & Wiklund, 2006) results in variation in meat quality depending on the amount of stress incurred by the animal ante-mortem (Wiklund *et al.*, 1995;

North *et al.*, 2016). Muscle pH_u is also linked to the appearance of meat with pH ≥ 6.06 in game species such as black wildebeest (*Connochaetes gnou*) resulting in meat that appears dark, firm and dry (DFD; Shange *et al.*, 2019).

The rate of pH decline post-mortem in relation to temperature of the carcass also plays a role in determining meat tenderness as it affects enzymatic processes linked to meat tenderisation (Hoffman *et al.*, 2007a; Matarneh *et al.*, 2017). The calcium-activated protease, µ-calpain, plays a chief role in post-mortem tenderisation (Koohmaraie, 1996; Feiner, 2006a). Calpain activity is promoted by higher temperatures, pH (6.2 – 7.0) and greater calcium ion concentration (Warriss, 2000; Feiner, 2006a). The inability of the sarcoplasmic reticulum to sequester calcium ions as a result of declining pH post-mortem results in increased activity of µ-calpain (Matarneh *et al.*, 2017). Springbok *Longissimus thoracis et lumborum* (LTL) muscles have been shown to have high temperature decay constants and low pH decay constants (North *et al.*, 2016). This indicates that pH of these muscles declines slowly because of rapid temperature decline rates because of the impact that temperature has on enzyme activity. Although generally, the rate of cooling of springbok carcasses post-mortem is high due to the small size of the carcasses (North *et al.*, 2016) as well as low to no levels of subcutaneous fat.

The pH of meat has an influence on its shelf-life (Wiklund *et al.*, 1995; Matarneh *et al.*, 2017), appearance (Hoffman *et al.*, 2007a; Shange *et al.*, 2019) and tenderness (Hoffman *et al.*, 2007a). Similar to meat from other species, the typical pH observed in springbok meat (5.4-5.5) (North & Hoffman, 2015; Neethling *et al.*, 2018) also falls within the range that microbial spoilage occurs (5.5 – 6.5) with higher pH increasing the likelihood of meat spoilage (Feiner, 2006b; Samelis, 2006; Matarneh *et al.*, 2017; Shange *et al.*, 2019). Both the pigments that are primarily responsible for meat colour, as well as the enzymes involved in meat tenderisation are protein in nature and their configuration and functionality is altered by pH (Faustman & Suman, 2017; Matarneh *et al.*, 2017). Considering the intricate relationship between pH and meat quality parameters explained above, the role pH plays in the various quality attributes will be highlighted throughout the review where applicable.

2.4 Ageing meat

Ageing, also referred to as conditioning, is a process that involves holding unprocessed meat above its freezing point without microbial spoilage occurring (Lawrie & Ledward, 2006). Ageing is usually done at temperatures ranging from zero to 10°C in a temperature-controlled room or an ageing chamber (Campbell *et al.*, 2001; Soriano *et al.*, 2016). Ageing is attributed to increasing tenderness and improving flavour and the overall eating quality of the meat as a result of various physiochemical changes that occur (Lawrie & Ledward, 2006). The vast majority of research on ageing methods pertains to beef and there is limited information on the effects of different ageing methods on springbok meat.

Depending on the method and duration of ageing, the quality of meat obtained can vary significantly. Some of the processes that occur during ageing continue to occur as long as conditions are favourable. Processes such as microbial activity can continue to proceed for as long as nutrients and favourable growth conditions are present. Consequently, differences in the length of and ageing conditions, will result in differences in meat quality.

Concerning ageing method, it can be theorised that the major difference between ageing methods that would arise is in the type of environment generated around the meat. If these environments differ, meat quality may differ. For example, in dry ageing where meat is exposed to the external environment, there will likely be a greater amount of moisture lost than in a process where a physical barrier, usually a vacuum bag, exists which protects the meat from the external environment (Jansen van Rensburg, 1997).

The two general ageing methods applied to meat are either wet ageing or dry ageing. Wet ageing refers to the treatment in which meat is aged in the presence of a physical, usually low gas permeable barrier between the meat and the external environment (Laster *et al.*, 2008; Kerry & Tyuftin, 2017). Wet ageing is done by vacuum sealing meat in a vacuum bag and ageing it under chilled conditions. Vacuum sealing produces an anaerobic environment that retards the growth of aerobic microorganisms on meat thus reducing the likelihood of spoilage during the process (Jones, 2004; Li *et al.*, 2013; Kerry & Tyuftin, 2017). Meat can be wet aged with or without bones depending on the cut and the producer's needs. Skin-on ageing of carcasses would likely fall under this category as the majority of the meat cuts are protected from the external environment by the skin.

Dry ageing is the second ageing method and is described as an ageing process where meat is exposed to the external environment during ageing (Laster *et al.*, 2008). Dry ageing is typically conducted in a temperature-controlled cold room or an ageing chamber designed for this specific purpose (Parrish *et al.*, 1991; Campbell *et al.*, 2001; Kim *et al.*, 2016; Soriano *et al.*, 2016). Meat may be dry aged on the carcass or off the carcass in order to save refrigeration space. Meat off the carcass can also be aged on or off the bone. Commercially dry aged meat has typically undergone a vacuum storage process before the ageing treatment as this is how most meat is transported (Campbell *et al.*, 2001). The exposure of meat to the environmental conditions increases the likelihood of microbial contamination especially from psychrophilic microorganisms; particularly in the early phase of the process when the external surface is still moist (Campbell *et al.*, 2001; Stenström *et al.*, 2014). Additionally, dry ageing results in crust formation due to greater moisture loss on the surface of meat that causes greater trimming losses (Parrish *et al.*, 1991). On the other hand, unique flavours associated with dry ageing have made it a consumer favourite (Parrish *et al.*, 1991; Kim *et al.*, 2016).

An intermediate method of ageing known as 'dry bag ageing' exists. This method combines the benefits of the two above-mentioned methods. Dry bag ageing makes use of a specially designed bag that has a high water vapour transmission rate in order to simulate dry ageing in this way while reducing crust formation and moisture loss as well as mitigating contact with the external environment (Li *et al.*, 2013; Stenström *et al.*, 2014; Prieto *et al.*, 2018).

2.4.1 Physical attributes

The physical attributes of meat play an important role in the consumption thereof as these are likely the first criteria consumers and retailers interact with when purchasing meat. Some important physical attributes in meat include colour, carcass and muscle weight and tenderness. These attributes also play a role in determining the price of the meat and are thus essential to producers.

2.4.1.1 Carcass characteristics

Carcass characteristics such as dressing percentage, weep loss and cooking loss can be important quality assessment parameters for both producers and consumers. Dressing percentage, the percentage ratio of the dressed cold carcass weight to live animal weight (Van Zyl & Ferreira, 2004), is an important indicator of potential income for the supplier. A higher dressing percentage means that a producer can generate more income through sales as generally carcasses are priced on a ZAR/kg basis. Similarly, weep and cooking loss can affect muscle weight thereby affecting the price of the product.

During skin-on ageing, dressing percentage can be expected to drop due to longer exposure of carcass surfaces to the cold room environment where evaporation occurs (FAO, 1991; Mallikarjunan & Mittal, 1998). In general a high relative humidity (~90%) and air speed of 0.5 m/s is recommended to prevent condensation forming on carcasses while minimising evaporation losses (FAO, 1991). The exposed muscles and skin (Balada *et al.*, 2008) have a high moisture content and are prone to dehydration during skin-on ageing. However, moisture lost from the skin can impact the final percentage weight lost during ageing but not the weight of the muscles covered by the skin. As in red deer (*Cervus elaphus*), there was no difference ($p \geq 0.05$) after 24 h in skinned carcass weight between carcasses aged skin-on and those aged skin-off (Soriano *et al.*, 2016). In the body cavity, the fillet is left exposed during skin-on ageing and resulting moisture and consequently weight loss. This is detrimental to the supplier as this muscle is typically sold as a prime cut and weight loss due to evaporation and trimming result in less income obtained (Laster *et al.*, 2008). However, in most cases, the neck and belly area, where the most moisture loss occurs, are areas of low value cuts or cuts that are typically disposed of during processing (Van Schalkwyk & Hoffman, 2016).

Weep loss (i.e. purge loss) is a quantification of the moisture lost from muscles during the storage or ageing process of meat. The decline of water holding capacity of muscles caused by declining pH results in increased weep loss (Matarneh *et al.*, 2017; Warner, 2017). In beef short loin steaks, interaction between ageing method and time were found to affect the purge loss ($p = 0.0155$) with the highest losses recorded in 14 and 28 day wet aged steaks and lowest loss in 14 day dry aged steaks (Smith *et al.*, 2008). Laster *et al.* (2008) reported similar findings in beef top sirloins with the higher ($p = 0.0115$) purge loss in 28 and 35 day wet aged steaks than both wet and dry 21 day aged steaks. Dry aged steaks overall experienced lower weep loss than wet aged steaks likely due to crust formation that occurs during dry ageing that limits the extent to which further evaporation can occur.

Percentage cooking loss is a quantifier of the percentage weight lost when a meat cut is cooked. As cooking loss is similarly linked to the water holding capacity of meat, changes to water holding capacity during ageing can affect the cooking loss (Warner, 2017). Dry aged beef steaks generally exhibit lower cooking loss than wet aged steaks (Dikeman *et al.*, 2013; Kim *et al.*, 2017; Oh *et al.*, 2018). Oh *et al.* (2018) found that cooking loss in dry aged beef steaks decreased with ageing time (2 vs. 28 days; $p < 0.0001$) while cooking loss in wet aged steaks did not differ ($p = 0.73$) with ageing time. Moisture loss due to evaporation during dry ageing has been reported as the cause of lower cooking loss in dry aged meat (Oh *et al.*, 2018). Wet aged springbok loins also exhibited no differences ($p = 0.132$) in cooking loss with ageing time which was attributed to a greater moisture loss across ageing days in the freeze-thaw process (Shanks *et al.*, 2002; North & Hoffman, 2015).

2.4.1.2 Colour

Myoglobin is the main protein in meat responsible for colour (Faustman & Suman, 2017). The concentration and state of myoglobin present in muscles impacts the colour of meat. Higher myoglobin content in the muscles of game animals causes game meat to appear darker than meat from farmed animals (Daszkiewicz *et al.*, 2009; Neethling, 2016b; Soriano *et al.*, 2016). Additionally, the redox state of myoglobin affects the colour of meat. Purplish red deoxymyoglobin can be oxidised to red oxymyoglobin (OMb) which can further be oxidised to brown metmyoglobin (MMb) (AMSA, 2012; Faustman & Suman, 2017).

The CIE L*a*b* system is a common way of measuring and describing meat colour. Colour in this system is measured along three axes which are L* (black 0 to 100 white), a* (−60 green to +60 red) and b* (−60 blue to +60 yellow) (AMSA, 2012). While L* is used as a measure of lightness in meat, a* and b* readings are further used to calculate hue angle and chroma values. Chroma is also known as the saturation index and gives an indication of the intensity of the colour being observed. Hue angle gives an indication of the specific colour being observed and can be used as an indicator of discolouration in meat over time (AMSA, 2012). During anaerobic storage of springbok LTL muscles a*, chroma values and % OMb decreased while hue angle values and % MMb increased with storage time (Neethling *et al.*, 2019). Correlations between these colour parameters and myoglobin in meat was established for springbok meat (Neethling, 2016b). The a* ($r = 0.75$, $p \leq 0.05$) and chroma ($r = 0.68$, $p \leq 0.05$) values correlated significantly with % OMb and can be used as an indication of concentration of % OMb or redness on the surface of meat. Additionally, the strong correlation established between % MMb and hue angle ($r = 0.83$, $p \leq 0.05$) further backs the use of hue angle as a measure of discolouration of meat (Neethling, 2016b).

The changes in meat colour during ageing is possibly due to the breakdown of myoglobin that occurs as time goes on. However, the extent of myoglobin denaturation that occurs is quite limited (Lawrie & Ledward, 2006). Differences in meat colour measurements because of ageing have been observed in meat from different species. There was an increase ($p < 0.05$) in L* values of wet aged beef for 28 days indicating that the meat was brighter in colour than 7 day wet aged beef (Ba *et al.*, 2014). Li *et al.* (2014) also found this increase ($p = 0.005$) in L* values for 19 day aged beef steaks compared to 8 day aged steaks, as well as an increase in hue angle values with ageing time across the ageing methods applied ($p < 0.001$). There was no significant impact of ageing method alone on colour measurements found in the same study.

In red deer, Soriano *et al.* (2016) found lower L* values, higher a* and b* values in meat from 3-day skin-on aged deer as compared to those aged for 1 day ($p < 0.05$). A sensory panel also reported that in the skin-on aged meat, 3-day aged meat had a darker red-brownish appearance compared to meat aged for 1 day ($p < 0.05$). A study comparing wet and dry ageing in beef reported lower L* ($p = 0.009$), a* ($p = 0.019$) and chroma ($p = 0.019$) values in dry aged loins than wet aged ones after three weeks of ageing (Kim *et al.*, 2016). The lower L* values indicate that the dry aged meat was darker in colour and this could be due to greater moisture loss during dry ageing that results in less light being reflected (Faustman & Suman, 2017). The a* and chroma values although statistically significant would likely not be noticeable as the difference in the measurements was quite small. Finally, hue angle was not significantly impacted by ageing method ($p = 0.233$) (Kim *et al.*, 2016).

2.4.1.3 Instrumental tenderness

The amount of force required to cut through a piece of meat is seen as a good indicator of meat tenderness. Warner-Bratzler Shear Force (WBSF) is a measure of the tenderness of meat with higher shear force values indicating lower tenderness values. One of the major benefits of ageing meat is the increase in tenderness that results from this process, due to the breakdown of myofibrillar components through enzymatic actions (Lawrie & Ledward, 2006). As discussed earlier, the calpain system is considered the initial protease responsible for post-mortem tenderisation with µ-calpain as the dominant enzyme in the process (Koohmaraie, 1996; Feiner, 2006a; Matarneh *et al.*, 2017). Tenderisation is thought to stem from the breakdown of long muscle fibres as a result of calpain action (Feiner, 2006a). Cathepsins are another class of enzymes linked to tenderisation that breakdown troponin-T and some collagen cross-linkages (Warriss, 2000). Due to the high optimal pH for calpain functioning, cathepsins (optimal pH range of 5.4 to 5.9), are hypothesized to play a role in further post-mortem tenderisation (Feiner, 2006a; Matarneh *et al.*, 2017).

Longer ageing periods have been found to result in more tender meat with lower WBSF values (Smith *et al.*, 2008; Ba *et al.*, 2014). Beef loins aged for 28 days had significantly lower WBSF values than those aged for 7 days (Ba *et al.*, 2014). This is consistent with studies on springbok and eland (*Taurotragus oryx*) meat that found a decrease in WBSF values with increased ageing time (North & Hoffman, 2015; Needham *et al.*, 2020). The shear force reported in springbok reduced from 23.26 N after one day of ageing, to 20.15 N after ageing for 28 days. This can be explained by the breakdown of myofibrillar and sarcoplasmic proteins by proteases that occurs as ageing proceeds (Lawrie & Ledward, 2006; Matarneh *et al.*, 2017).

Jansen van Rensburg (1997) found some significant differences in shear force readings due to ageing method and time used. In a study where four different ageing methods were applied on three age groups of springbok, deboned and vacuum packaged aged meat from young animals was found to have lower ($P < 0.05$) shear force values than bone-on vacuum packaged aged meat from older animals. However, the influence of age of animals should be noted, as there was also a significant interaction reported between animal ages and ageing method used. There were no differences ($P > 0.05$) reported with the other two ageing methods (skin-on and skin-off, on-carcass). Skin-on ageing, however, tended to produce meat with lower shear force values than any of the other ageing methods (Jansen van Rensburg, 1997).

There have also been varying results regarding the significance of effects of ageing method on instrumental tenderness of beef. Some research reports no significant effect of ageing method on shear force (Dikeman *et al.*, 2013; Kim *et al.*, 2016) while others have found significant differences (Laster *et al.*, 2008; Smith *et al.*, 2008). Laster *et al.* (2008) found that wet aged steaks had significantly lower shear force values that the dry aged ones while Dikeman *et al.* (2013) and Smith *et al.* (2008) found dry aged steaks had lower, although not significant, shear force values than wet aged ones. It is possible that these differences resulted from inherent variation between the animals or muscles analysed.

2.4.2 Microbiological attributes

The process of ageing can result in changes in the microbial content of meat as microorganisms are still able to grow in an ageing environment (aerobic and/or anaerobic), albeit at a slower rate due to the low temperatures employed. The exponential nature of microbial growth with time means that not only does ageing time have an effect on microbial quality, but there also exists a limit to which ageing is applicable before microbial quality becomes a limiting factor. Some extent of microbial activity could be desirable for improving meat flavour but excessive activity can result in spoilage of meat. For example, growth of lactic acid producing bacteria (LAB) can increase shelf-life by lowering the pH of meat through lactic acid production but also produces undesirable flavours (Pothakos *et al.*, 2015). Therefore, ageing time is an important aspect in determining the end microbial quality of aged meat.

In both dry aged and wet aged beef loins, samples aged for three and five weeks were found to have higher plate counts ($p < 0.05$) for aerobes, coliforms and anaerobic organisms than samples aged for one and two weeks (Newsome *et al.*, 1984). The microbial counts further increased during retail storage of the meat cuts. Similar results were found in dry aged beef loins where a notable increase was reported in the first two weeks of ageing followed by a decrease in rate of microbial growth thereafter (Hulánková *et al.*, 2018). This later plateauing of growth was attributed to the loss of surface moisture required for survival of microorganisms that occurred during ageing.

Different ageing methods can produce different environments that may be more or less favourable for particular organisms to grow. For example, the anaerobic environment produced while ageing meat in a vacuum bag inhibits growth of strict aerobes but also facilitates the growth of anaerobic organisms such as LAB (Kerry & Tyuftin, 2017). Dry ageing methods (an aerobic environment) do not allow for the growth of anaerobic organisms (Zagorec & Champomier-Vergès, 2017). Springbok loins have been found to have higher LAB counts in samples that were aged deboned in vacuum packaging than those aged skin-off while on the carcass (Buys *et al.*, 1997), although, the differences in total microbial counts resulting from ageing method applied were not significant ($p = 0.5390$) (Buys *et al.*, 1997). Low LAB counts have also been reported in dry aged meat (Hulánková *et al.*, 2018).

Nonetheless, the ageing method applied should not compromise the microbial safety of the final product. It is expected that dry aged meat would have much higher microbial counts as the entire surface of the meat is exposed to the environment for the duration of the ageing process. However, studies show that microbial counts during dry ageing that were still within acceptable limits for human consumption (Campbell *et al.*, 2001; Soriano *et al.*, 2016; Hulánková *et al.*, 2018). In addition to this, the counts were well below the typical limit, ca. 7 log CFU per cm^2 or gram, for development of off flavours associated with microbial spoilage (Hulánková *et al.*, 2018). Newsome *et al.* (1984) found that vacuum packaging was not able to inhibit the growth of aerobes as there were no reported differences in aerobic plate counts between the dry ageing treatment applied and ageing in the vacuum packaging. This however could be as result of ineffective vacuum sealing during the trial that meant the environment was not completely anaerobic. Buys *et al.*(1997) found an inhibition of *Enterobacteriaceae* growth in springbok loins after ageing for 12 days in vacuum packaging unlike with skin-on and skin-off ageing where growth continued to increase.

Skin-on ageing poses a microbiological threat as the skin is notably one of the main surfaces from which cross contamination of meat during skinning can occur (FAO, 1991). Bell (1997) found that a high potential for contamination occurred at carcass opening cut sites and sites that could come in contact with the skin during skinning. Additionally the aerobic environment surrounding the carcasses during ageing facilitates the growth of spoilage organisms such as *Pseudomonas spp.* during ageing (Nortjé & Shaw, 1989). Hygienic skinning practices should therefore be at the forefront especially when dealing with skin-on aged carcasses.

2.4.3 Chemical attributes

2.4.3.1 Proximate composition

Meat is primarily composed of moisture, protein, fat and ash; referred to as proximate composition. Processes such as lipid oxidation, evaporation and proteolysis that can occur when meat is stored could potentially affect the proximate composition of meat. However, ageing time was found to have no effect on proximate composition of red deer (Soriano *et al.*, 2016). Although, the study was done on a shorter time scale, 3 days, than is typical for venison and beef ageing times (Jansen van Rensburg, 1997; North & Hoffman, 2015; Needham *et al.*, 2020). Application of longer ageing times has yielded significant differences in moisture and fat content with the former decreasing over time due to an increase in weep loss (Jansen van Rensburg, 1997; North & Hoffman, 2015). It is therefore likely that shorter ageing times simply do not allow for significant changes to proximate composition. Protein and ash content did not vary greatly as a result of either ageing method or time (North & Hoffman, 2015; Soriano *et al.*, 2016). Moisture and fat content however are affected by ageing time and method (Jansen van Rensburg, 1997; North & Hoffman, 2015). It should also be noted that moisture content is thought to affect the colour of meat (Kim *et al.*, 2016) whilst fat affects the amount and type of volatile compounds produced (Frank *et al.*, 2016).

In beef, no significant effect of ageing time on moisture content of wet aged steaks has been found (Ba *et al.*, 2014; Holman *et al.*, 2019). Moisture loss is attributed to evaporation during the ageing period. An ageing method such as dry ageing where a larger surface area is exposed to the environment results in a greater evaporation rate and thus lower moisture content than in wet ageing where the surface area exposed to the environment is limited. However, Dikeman *et al.* (2013) found significantly higher percentage moisture in wet aged steaks than dry aged and special bag aged steaks. Cutting muscles has also been suggested to increase likelihood of moisture loss from the cut surfaces where moisture originating from the inter-muscular spaces is expelled due to decreased water holding capacity (Warriss, 2000). This process is the source of the drip observed in the vacuum packaging at the end of ageing.

Intramuscular fat (IMF) percentage decreased ($p = 0.024$) with ageing time in cooked aged springbok loins with lower IMF content recorded after 28 days than 1 and 3 days of ageing (North & Hoffman, 2015). In springbok meat, deboned loins that were aged in vacuum packaging were found to have significantly higher total fat percentage than loins that were aged skin-on, skin-off on-carcass and in vacuum packaging with bones still present (Jansen van Rensburg, 1997). In beef, raw dry aged steaks were found to have higher fat percentage than wet aged and steaks aged in special ageing bags that simulate dry ageing ($p = 0.04$); the wet aged cooked steaks similarly had higher percentage fat ($p = 0.04$) than dry aged or special bag aged steaks (Dikeman *et al.*, 2013). One reason suggested for the drop in IMF content was a loss in physical structure with ageing time due to tenderisation that allowed more IMF to be lost as drip during the cooking process (North & Hoffman, 2015). However, this loss was not reflected in the cooking loss results for both springbok ($p = 0.132$) (North & Hoffman, 2015) and beef (Dikeman *et al.*, 2013). It is also possible that the differences in fat content reported arose from differences in moisture reported; the nature of proximate composition analysis implies that decrease in percent distribution of one component will result in percent increase of the other components.

2.4.3.2 Fatty acid profile

There are two essential fatty acids, linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3), that cannot be synthesized in the human body and are the basis for formation of longer chain fatty acids (Fig. 2.1) (Sprecher, 1992; Yehuda, 2009). Long chain fatty acids are further metabolised in the body to produce regulatory compounds. In general, meat has been an important source of essential fatty acids in the human diet. However due to the increased reliance on omega-6 (n-6) rich diets in meat production, omega-3 (n-3) consumption has declined while n-6 consumption has increased (Sanders, 2000; Watson, 2009). The intake ratio of these fatty acids has since become the focus as they are metabolised in the same pathway (Fig. 2.1) and compete for metabolic enzymes (Commission of European Committees, 1992; Sprecher, 1992). The recommended n-6:n-3 intake ratios differ with regards to potential health benefit (Simopoulos, 2004) however in general, reducing dietary n-6 intake while increasing n-3 intake is recommended. The fatty acid profile is integral in the perception of game meat as a healthy product for consumers as not only does springbok meat contain appreciable amounts of unsaturated fatty acids, it also contains them in the desirable PUFA:SFA and n-6:n-3 ratios (Hoffman *et al.*, 2007c; Neethling *et al.*, 2018).

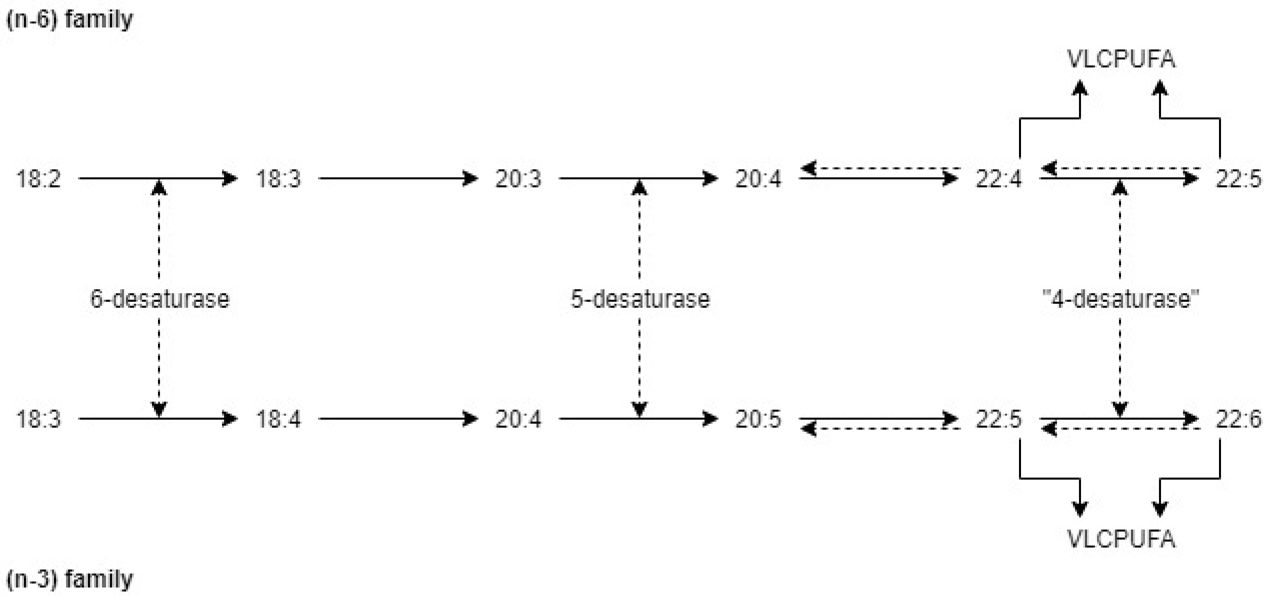

Figure 2.1 Schematic of microsomal metabolic pathways of linoleic acid and α-linolenic acid adapted from Sprecher (1992). Dashed lines indicate retro-conversion C22 fatty acids to C20 fatty acids. VLCPUFA - Very long chain PUFA

Aside from the nutritional value, the fatty acid profile also plays a determining role in flavour development in meat. Differences in fatty acids can result in production of different aroma compounds on cooking. Some of the changes that occur to aroma and flavour during ageing have been attributed to the changes in the free fatty acid composition of meat (Wood *et al.*, 2003; Lawrie & Ledward, 2006). Various fatty acids have also been linked to the production of specific aroma compounds. For example, 1-pentanol, hexanal, 2,4-decadienal and heptanal are some of the volatile compounds present in meat that are formed from the oxidation of linoleic acid while 1-penten-3-ol and benzaldehyde are associated with oxidation of α-linolenic acid (Elmore *et al.*, 2002). The rate at which these fatty acids are oxidised during ageing can therefore have a noticeable effect on the flavour and aroma development in meat. Higher rates of oxidation have been linked to production of a greater number of volatile compounds (Frank *et al.*, 2016).

There is a higher risk of rancidity developing when the majority of the fatty acids present in game meat are unsaturated (Wood *et al.*, 2003). Rancid flavours develop as a result of oxidation and cause shorter shelf-life for meat. Thiobarbituric acid-reactive substances (TBARS) is generally used as a measurement of lipid oxidation; 21 day aged beef had significantly higher TBARS values than fresh beef (Sosin-Bzducha & Puchała, 2017). Similarly, beef loins aged for 28 days had higher TBARS values than those aged for 7 days indicating greater lipid oxidation occurs with longer ageing periods (Ba *et al.*, 2014). A mean TBARS value of 0.5 mg MA/kg is seen as the limit at which consumers have reported rancid flavour (Wood *et al.*, 2008). The increase in lipid oxidation can be associated with the increase in by-products of this process thereby accounting for changes in flavour perceived with increasing ageing time.

During ageing of beef, changes to capric (C10:0) ($p = 0.03$), palmitoleic (C16:1) ($p = 0.04$), stearic (C18:0) ($p \leq 0.01$) acids and conjugated linoleic acid (CLA t-9t-11) ($p = 0.04$) were found. Capric and palmitoleic acids increased after 21 days of ageing while stearic and CLA t-9t-11 decreased (Sosin-Bzducha & Puchała, 2017). The ratio of n-6 to n-3 PUFAs was higher, although not significantly so, after 21 days of ageing compared to 2 days ($p = 0.22$). Attack of unsaturated fatty acids by free radicals results in oxidation and break down of fatty acids. The effect of ageing method and time on fatty acid profile in springbok meat has not as of yet been investigated. Considering the importance of fatty acid profile on both the nutritional and eating quality of springbok meat, as well as the potential effect of ageing on fatty acid profile, monitoring changes to fatty acid profile of springbok meat during ageing may play a key role in understanding changes in springbok meat flavour and thus producing meat with consistent quality.

2.4.3.3 Volatile compound profile

Aroma is the most important component of flavour (Lawrie & Ledward, 2006). The mixture of volatile compounds present play an important role in aroma and flavour perception of foods (McGorrin, 2012; Watanabe *et al.*, 2015). These volatile compounds can arise as a result of several processes occurring during the storage, ageing and cooking of meat (Lawrie & Ledward, 2006; Flores, 2017). Ageing meat also facilitates the production of compounds that are precursors to the volatile compounds produced during cooking of meat. Lipid oxidation and fatty acid degradation during ageing as well as thermal oxidation and Maillard reactions during cooking are the main processes thought to be responsible for the production and release of major volatile compounds (Flores, 2017). It is therefore expected that volatile compounds produced can vary depending on the fatty acid composition of meat resulting from the time and method of ageing applied (Ba *et al.*, 2014; Watanabe *et al.*, 2015).

Longer ageing periods can cause a decrease in concentration of the desirable volatiles that decreases the overall flavour quality of meat (Ba *et al.*, 2014). The volatiles affected by the ageing time are mainly those associated with lipid oxidation; compounds such as 2-pentyl furan, toluene and 2,4-decadienal that form as a result of lipid oxidation increase in concentration with increase in ageing days (Ba *et al.*, 2014; Watanabe *et al.*, 2015). The total ketones and volatile compounds were higher ($p \leq 0.05$) after 6 days of ageing ($p \leq 0.05$) while total aldehydes were significantly higher after 14 days of ageing in foal meat ($p \leq 0.05$) (Maggiolino *et al.*, 2018). The majority of the volatile compounds observed were aldehydes which have been noted to have a low odour threshold (Elmore *et al.*, 1999; Wood *et al.*, 2003) and therefore can be said to contribute significantly to perceived flavour. Of the aldehydes produced, hexanal was the most abundant and increased in concentration with ageing time.

In foal meat, of the 116 volatile compounds identified, only 12 were affected by ageing time (Maggiolino *et al.*, 2018). The volatile compounds that arise because of ageing of meat are far too numerous to extensively cover in this review therefore only a few of the key volatiles that have also previously been identified in springbok (Neethling, 2016a) will be discussed.

Ba *et al.* (2014) reported that hexanal along with 2-octenal and 2,4 decadienal were produced as a result of oxidation of linolenic acid. Other significant volatiles that were found to vary with ageing time in foul meat included toluene ($p = 0.004$), benzaldehyde ($p = 0.018$), octanal ($p = 0.021$), nonanal ($p = 0.005$) and acetoin ($p = 0.01$). Save for acetoin, the rest of the reported volatiles are reported products of lipid oxidation that proceeds during ageing. The evidence presented thus far suggests that volatile compound production during ageing is closely linked to oxidation of fatty acids present.

Similar to the fatty acid profile, there is a lack of information on the influence of ageing method and time on the profile of volatile compounds in springbok meat. Nonetheless, particular changes to the flavour profile of aged meat suggests that ageing method does have an effect on the volatile compound profile. Dry aged beef was found to be more flavourful than wet aged beef ($p = 0.016$) (Kim *et al.*, 2016) and the intensity of flavour has been linked to the amount of volatile compounds produced (Ba *et al.*, 2014). Consumers in this study also noted that the dry aged beef had more a beefy/roasted flavour compared to wet aged beef. This was attributed to the greater abundance of metabolites such as glutamate which are associated with beefy flavour (Kim *et al.*, 2016). Changes in compounds such as nonanal that are associated with beef flavour (Moon *et al.*, 2006) could possibly also be the cause of the above-mentioned difference in beefy flavour. Other studies have found variation in flavour intensity as well as other flavour and aroma attribute scores in game meat with ageing method (Jansen van Rensburg, 1997; Soriano *et al.*, 2016) suggesting that changes to the volatile compound profile with ageing should be investigated.

2.5 Sensory attributes

The flavour of meat is influenced and generally improved by the process of ageing (Flores, 2017). The changes during ageing explored thus far have knock-on effects on the subsequent perception of flavour and texture of meat. It can therefore be expected that ageing method and time would affect the sensory attributes of meat.

Previously reported sensory attributes during Descriptive Sensory Analysis (DSA) of fresh and aged springbok meat and their descriptors (North & Hoffman, 2015; Neethling *et al.*, 2018) are listed in Table 2.3. The two studies made use of reference samples specific to meat in general (e.g. beef loin, ox liver, lamb loin and chicken breast) and game meat in particular [e.g. springbok, Egyptian goose (*Alopochen aegyptiacus*), blesbok (*Damaliscus pygargus phillipsi*) and fallow dear (*Dama dama*)] to calibrate sensory panellists on the 100-point line scale (North & Hoffman, 2015; Neethling *et al.*, 2018). Once references were established and panel training was completed via the ballot and consensus method, testing of samples followed via the test retest method where samples were scored for each of the attributes on an unstructured line scale from 0-100.

Table 2.3 The sensory attributes and descriptors associated with fresh and aged springbok meat*

Sensory attribute	**Description**
Overall aroma	Intensity of the overall aroma in the first few sniffs
Gamey aroma	Aroma associated with meat from wild animal species – sometimes a combination of liver-like and metallic aromas
Beef-like aroma	Aroma associated with cooked beef loin
Metallic aroma	Aroma associated with raw meat/blood-like
Liver-like aroma	Aroma associated with pan-fried beef ox liver
Sweet-associated aroma	Aroma associated with the browning of a cooked meat surface (Maillard reaction)
Sour aroma	Aroma associated with vacuum packed aged meat/off meat
Off/manure aroma	Unpleasant aroma associated with farmyard/contamination/off meat
Gamey flavour	Flavour associated with meat from wild animal species – sometimes a combination of liver-like and metallic flavours
Beef flavour	Flavour associated with cooked beef loin
Metallic flavour	Associated with raw meat or a blood-like taste
Liver-like flavour	Flavour associated with pan-fried beef ox liver
Sour flavour	Flavour associated with off milk
Sweet taste	Taste associated with a sucrose solution
Salty taste	Taste associated with sodium ions
Initial juiciness	Amount of fluid extruded on surface of meat when pressed between thumb and forefinger (perpendicular to fibres)
Sustained juiciness	Amount of moisture perceived during mastication
Tenderness	Impression of tenderness after mastication
Residue	Residual tissue remaining after mastication (difficult to chew through)
Mealiness	Disintegration of muscle fibres into very small particles (perception within the first few chews)
Liver-like texture	Texture similar to that of pan-fried beef ox liver (spongy/pasty)

*Adapted from North, 2014 and Neethling *et al.*, 2018

2.5.1 Aroma and flavour

The importance of aroma and the process attributed to its formation during ageing have previously been discussed (Sections 2.4.3.2 and 2.4.3.3). Gamey aroma and flavour attributes have been linked to PUFA content of springbok (Hoffman *et al.*, 2007d) and reindeer (*Rangifer tarandus*) (Swanson & Penfield, 1991) meat. Descriptors such as “sweet”, “fruity”, “sour” and “putrid” have been used for meat associated with microbial spoilage (Borch *et al.*, 1996; Feiner, 2006b; Lawrie & Ledward, 2006). This section will explore the differences in aroma and flavour attributes reported during descriptive sensory analysis of aged meat.

In red deer, aroma and flavour intensity, gamey and sweet/caramel aromas and flavours were found to be higher in skin-on aged samples when compared to those aged skin-off on-carcass ($p \leq 0.05$) (Soriano *et al.*, 2016). Furthermore, lower metallic flavour was detected in in meat from skin-on aged deer compared to meat from skin-off on-carcass aged deer. Higher concentrations of certain volatile compounds that are associated with “pleasant” aromas result in higher overall flavour scores (Ba *et al.*, 2014). In springbok, the only difference in aroma scores linked to ageing method was an interaction between animal age and ageing method ($p = 0.002$) (Jansen van Rensburg, 1997). However, post-hoc testing with the Bonferroni difference test to reveal which particular scores differed significantly did not detect any significant differences. Differences in aroma scores due to ageing method alone were not significant ($p = 0.755$).

In DSA of aged springbok meat no difference in overall aroma scores ($p = 0.202$) with ageing time was found although scores generally increased with ageing time (North & Hoffman, 2015). Another study on ageing springbok meat reported increases in aroma and overall flavour with ageing time; aroma was higher after 14 and 21 days of ageing than after 3 days ($p = 0.001$) while overall flavour also increased with ageing time and was highest after 21 days of ageing ($p < 0.001$) (Jansen van Rensburg, 1997). In DSA of beef, non-significant increases in sensory scores for positive attributes (taste intensity and desirability) up to 14 ageing days was noted followed by a decline in these scores at 21 days (Sosin-Bzducha & Puchała, 2017). Along with these desirable aroma and flavour attributes, changes to the negative attributes were observed with increase in ageing time. Sour/aged aroma and flavour was higher ($p < 0.05$) in 28-day aged springbok. Metallic aroma and flavour, liver-like flavour and off/manure flavour were also higher ($p < 0.05$) after 28 days of ageing than 3 or 8 days of ageing. Increased intensity of these negative attributes suggesting that the 28-day ageing period may be too long for springbok meat (North & Hoffman, 2015).

2.5.2 Texture

Texture attributes of meat assessed during DSA by trained panellists include initial juiciness, tenderness, mealiness and residue. These attributes are influenced by numerous factors including IMF and muscle fibre structure (Hopkins, 2017). Texture is affected by ageing time due to the changes to structural proteins that occur during ageing as mentioned in section 2.4.1.3 (Lawrie & Ledward, 2006; Hopkins, 2017). Denaturation of myofibrillar and sarcoplasmic proteins also affects the perceived texture of meat. The total fatty acids present and IMF content in general also have an influence on tenderness and juiciness (Wood *et al.*, 2003; Hopkins, 2017).

Tenderness and residue of springbok meat were found to differ as a result of ageing time; tenderness scores increased with ageing time ($p = 0.001$) while residue decreased ($p < 0.001$) (North & Hoffman, 2015). Consumers would be assumed to experience these changes as positive (Neethling *et al.*, 2018), which supports the case for ageing of springbok meat. Eight and 28 days of ageing resulted in better tenderness and residue scores in springbok as compared to 1 and 3 ageing days (North & Hoffman, 2015). Jansen van Rensburg (1997) also reported an effect of ageing time on tenderness of springbok loins ($p < 0.001$). In that study, 3 and 7 days of ageing were reported to yield the ideal tenderness and the 14 days aged samples scored lowest for tenderness. However, the 14 day aged samples also had lower texture scores ($p < 0.001$) and were described as being "slightly mushy". Three and 21 days of ageing resulted in the most ideal texture scores.

Sustained juiciness also increases with ageing time. Twenty-eight day aged springbok scored higher ($p = 0.023$) for sustained juiciness than 1 and 3-day aged samples (North & Hoffman, 2015). In another study, ideal juiciness scores for springbok loins were reported from skin-off on carcass aged and bone-in vacuum pack aged loins aged for 3 days while deboned loins aged in vacuum packaging for 3 and 14 days had much lower scores ($p = 0.042$) and were described as "moderately dry" (Jansen van Rensburg, 1997). In beef, ageing method was reported to have no effect on juiciness (Parrish *et al.*, 1991; Kim *et al.*, 2016) for wet and dry aged loins ($p > 0.01$ and $p = 0.245$, respectively). This was attributed to crust formation during dry ageing that limited further evaporation. Similar findings were reported with no differences in juiciness scores of red deer between skin-off on-carcass aged meat and skin-on aged meat (Soriano *et al.*, 2016) likely since in both treatments, muscles were still attached to the carcass therefore result similar proteolytic activity in both ageing methods.

2.5.3 Consumer analysis

Understanding consumer attitudes and preferences towards game meat is essential in informing the choices made by producers and retailers concerning processing method. Sensory characteristics were identified at the main attribute that could change non-consumers of game meat into consumers (Wassenaar *et al.*, 2019). While large scale studies on consumer attitudes towards game meat in the western cape of South Africa (Hoffman *et al.*, 2005) and across South Africa (Wassenaar *et al.*, 2019) have been carried out, a study on consumer preferences of game meat in South Africa has yet to be done. Most conclusions drawn in the industry are based on domesticated red meat species which have a different flavour profile from game meat (Rødbotten *et al.*, 2004). As no such data exists for game in South Africa, the rest of this discussion will focus on the effect of ageing method and time on consumer preference for beef as few processors age lamb/mutton.

Consumers indicated awareness of the ageing process and consider it a positive term (Smith *et al.*, 2008). The same consumers indicated not being aware of dry ageing as a method of ageing and could not distinguish between wet and dry aged beef steaks pertaining to degree of liking of flavour, tenderness, juiciness and overall liking. Consumers also found no differences in level of tenderness and juiciness. The level of beef flavour however did differ ($p < 0.05$) between both wet and dry aged US choice grade steaks and wet aged US select grade steaks. The wet aged US choice steaks scored higher for level of beef flavour than both wet and dry aged US select grade steaks. A study with beef steaks from different cuts yielded similar results (Laster *et al.*, 2008) with consumers in this study reporting no difference scores for degree of liking for juiciness and flavour as well as level of beef flavour, juiciness and tenderness with ageing method. They however reported liking the tenderness of the dry aged steaks more than that of wet aged ones ($p = 0.0361$). An interaction for degree of liking for overall flavour between ageing method and ageing period was also established ($p < 0.05$); consumers preferred the flavour of 14 day dry aged steaks less than 35 day dry aged steaks and 14 day wet aged steaks.

Ageing time had an effect on the degree of liking for juiciness ($p = 0.0030$) as well as the level of beef flavour ($p = 0.0101$) and juiciness ($p = 0.0198$) in Smith *et al.*'s (2008) study. Samples aged for 28 days scored significantly lower than 35 and 21 day aged samples for liking of juiciness whilst 35 day samples scored higher than 28 day samples for level of juiciness. The 21 day samples scored higher than all the other samples for level of beef flavour (Smith *et al.*, 2008). On the other hand, Laster *et al*. (2008) reported no significant effect of ageing time on the degree of liking of the sensory attributes by consumers.

After assessing changes in beef quality due to ageing method with DSA, Dikeman *et al.* (2013) stated that consumers would likely not pick up on the differences observed by the trained sensory panel. They attributed this to few minor differences noticed between meat from the three ageing methods (dry, VAC and special bag ageing) by the trained panel. From the two consumer studies discussed (Laster *et al.*, 2008; Smith *et al.*, 2008), it would seem that consumers are able to pick up differences in textural attributes and overall flavour in beef; both of which are impacted by ageing. Even though consumers may not for example be able to notice differences in gamey aroma in springbok meat, gamey aroma may greatly contribute to the overall aroma that consumers are more likely to notice.

2.6 Game carcass handling methods in South Africa

The free market nature of the game industry in South Africa allows for various sources of game meat to enter the supply chain (Hoffman *et al.*, 2004). Although various guidelines and regulations have been set up with regards to hunting and processing of game carcasses (Van Schalkwyk & Hoffman, 2016; VPN/08/2017-01), there is limited information available on the optimal carcass handling procedures to ensure product uniformity for the consumer. This has allowed different carcass handling practices to be implemented based on the needs of the supplier further contributing the variation in meat quality (Jansen van Rensburg, 1997; Schack *et al.*, 2016).

The process of acquiring of animals for the meat production can generally be broken down into harvesting, evisceration, chilling and transportation for further processing (Directorate of Veterinary Services, 2007; Van Schalkwyk & Hoffman, 2016). During harvesting, animals are shot with either head or high neck shots by a professional marksman with a light calibre rifle. Such shots result in minimum ante-mortem stress to animals, allow for instantaneous death, and thus are considered more humane (Van Schalkwyk & Hoffman, 2016). Springbok are typically harvested at night with the aid of strong spotlight that temporarily blinds the animals allowing for more accurate shot placement and reduced risk of maiming, missed shots and frightening the herd (Conroy, 2005; Van Schalkwyk & Hoffman, 2016). The animal's throat is slit soon after death, severing the main jugular vein and carotid artery enabling sufficient exsanguination to occur once the animal is hang (Department of Agriculture, 2000; Van Schalkwyk & Hoffman, 2016).

The next step is transporting the carcasses back to either a field depot or an abattoir for skinning and evisceration. In cases where abattoirs are considerably far from the harvesting grounds, evisceration may be carried out in the field and the carcasses transported to the field depot for the health inspection before being loaded into chiller trucks for transportation back to the abattoir skin-on (Van Schalkwyk & Hoffman, 2016). Carcasses are also investigated by a state veterinarian or meat inspector to ensure that none of the animals are diseased (Directorate of Veterinary Services, 2007). Inspectors also survey carcasses for excessive contamination in the abdominal cavity as well as thoracic shots that can result in gross contamination. As most of the culling occurs some distance from the processing facility, most carcasses will have entered rigor mortis by the time they arrive at the facility. It is therefore important to ensure that the carcasses are loaded and transported in such a manner that the important muscles such as the LTL muscles are stretched to ensure more tender meat (Hopkins, 2017). Once the carcass has arrived at the processing plant/field depot, variation exists in the ideal skinning time for carcasses. Some processors prefer to skin the carcass before chilling whilst others prefer to chill carcasses skin-on. Irrespective of the process, skinned carcasses should never be hung in the same chiller with skin-on carcasses as the outer surface of the skin is a source of microbes (Bell, 1997) and could result in cross-contamination onto the skinned carcasses. After cooling down the carcass for 24 h post-mortem, carcasses are then aged. Meat can be aged in different ways (Table 2.4). The most obvious differences in the methods is that some recommend skinning the carcass and vacuum sealing the meat for the duration of the ageing period and others recommend keeping the meat on the carcass with the skin still mostly intact and acting as a barrier between the meat and the external environment. Some methods recommend skinning the carcasses and leaving the meat on the carcass for the duration of the ageing period (Table 2.4). As seen in Table 2.4, the optimal ageing time suggested also varies.

Table 2.4 Ageing methods and times recommended for different small game and game species

Species	Ageing method	Ageing time	Reference
Springbok	Skin-on in 4°C cold room	3-10 days	Jansen van Rensburg (1997)
Springbok	Vacuum packed in 4°C cold room	8 days	North & Hoffman (2015)
Whitetail deer	On-carcass, skin-off in 3°C cold room	1-3 days	Marchello *et al.* (1985)
Game species	On-carcass, skin-off in 0-4°C cold room	5-10 days	Schack *et al.* (2016)
Game species	Skin-on	7 days	Jansen van Rensburg (1997)
Game species	Skin-on	14-28 days	Jansen van Rensburg (1997)

One major source of variation that results in the implementation of different carcass ageing methods is the scale of the operation. Typically in commercial settings, game carcasses can be deboned three to seven days post-mortem (North, 2014). This time can be seen as the ageing period as carcasses are kept at conditions stipulated for ageing (Lawrie & Ledward, 2006). However, from a logistical point of view, leaving whole carcasses hanging in the cold room for the duration of the ageing period reduces turnover times as over-crowding the chiller restricts proper airflow (FAO, 1991). In addition to this, only prime cuts are worth ageing as most of the other cuts end up in processed products that do not necessarily benefit from the increase in tenderness that occurs during ageing. A solution to these shortcomings is extracting cuts that require ageing and ageing them either in vacuum packaging (wet ageing) or as dry aged cuts. This not only allows for faster turnover times but also potentially reduces the amount of moisture lost due to evaporation if the carcass had been left exposed in the cold room (in the case of wet ageing) (Hodges *et al.*, 1974).

2.7 Conclusion

It is clear to see that there is not one ageing method that stands out if the goal is to set a universally applicable standard; the different ageing regimens investigated in this review have both advantages and disadvantages. As consistency in quality is key to fostering growth of the game meat industry in South Africa, there is a need to understand how ageing methods and time affect the meat quality of game species. Additionally, the needs of both the suppliers and consumers of game meat need to be understood and considered when choosing an ageing method and time.

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CHAPTER 3

The effects of ageing method and time on microbiological quality, physical and proximate composition of Springbok (*Antidorcas marsupialis*) *Longissimus thoracis et lumborum* muscles

3.1 Abstract

This study determined the effect of ageing method [skin-on and vacuum bag ageing (VAC)] and ageing time (6, 7, 8 and 9 days) on the physical and chemical composition and microbiological quality of the *Longissimus thoracis et lumborum* muscles of 24 springbok (12 males and 12 females). Skin-on aged carcasses lost on average 5.2% of their weight during the ageing period. On day 6, skin-on aged samples had higher a* ($p = 0.050$) and chroma ($p = 0.035$) values than the VAC aged samples. Warner-Bratzler shear force was higher ($p = 0.040$) in 7 day skin-on aged samples than on any other ageing day or ageing method. Female springbok exhibited higher intra muscular fat content and cooking loss ($p = 0.004$ and 0.028, respectively) than male springbok, whilst the moisture content was higher in males ($p = 0.016$) than females. Ageing time affected the hue angle ($p = 0.037$) and b* ($p = 0.028$) similarly with day 6 aged samples exhibiting lower values than day 7 and 9 samples. Skin-on aged samples had higher percentage cooking losses ($p = 0.019$) and lower Lactic Acid Bacteria counts ($p = 0.102$) than VAC aged samples. Additionally, aerobic plate counts ($p = 0.094$) as well as ultimate pH ($p = 0.013$) increased with ageing time after 7 days. None of the samples tested positive for *E. coli* and coliforms. The ageing methods and times previously recommended for springbok meat were able to produce microbiologically safe meat with few differences in physical attributes examined.

Keywords: Game meat, skin-on ageing, vacuum bag ageing

3.2 Introduction

The practice of ageing meat has long been applied to improve the tenderness and flavour thereof. Traditionally meat was aged on carcass (with or without skin-on), however, with the advent of vacuum packaging, the latter has become the preferred methodology (Campbell *et al.*, 2001). Ageing has been shown to affect colour (Lawrie & Ledward, 2006), moisture content (Dikeman *et al.*, 2013; Oh *et al.*, 2018) and overall carcass yield (Parrish *et al.*, 1991; Kim *et al.*, 2016). As the appearance of game meat was highlighted as one of the three key physical characteristics influencing consumer choice (Hoffman *et al.*, 2004; Wassenaar *et al.*, 2019), and appearance (Hoffman *et al.*, 2004; Neethling *et al.*, 2019), whilst cost efficiency (Parrish *et al.*, 1991) is of concern to the supplier, it is necessary to take these factors into account when ageing meat. Additionally, improving the quality of a meat product should not come at the expense of microbial safety and quality.

Game meat has long been perceived by consumers as dark, dry and tough (Radder & Grunert, 2009; Schack *et al.*, 2016) although recent studies have shown that the latter is not the case with game, particularly springbok, being more tender than meat from conventional livestock species (Jansen van Rensburg, 1997; North & Hoffman, 2015; Neethling *et al.*, 2018). Ageing of venison/game meat has been linked to improvement of tenderness (Lawrie & Ledward, 2006; Soriano *et al.*, 2016) although in game meat, the main benefit of ageing is in both textural and flavour improvement (North, 2014; Schack *et al.*, 2016). There are three major ageing methods applied in the meat industry i.e. dry ageing, wet ageing and special bag ageing (Campbell *et al.*, 2001; Dikeman *et al.*, 2013; Li *et al.*, 2014; Stenström *et al.*, 2014). In wet ageing, meat is vacuum packed in a vacuum bag that creates an anaerobic environment around the meat. Special bag ageing simulates a dry ageing environment while still providing protection to the meat during ageing to prevent environmental contamination (Li *et al.*, 2013). Dry ageing however occurs either in a temperature controlled room or an ageing chamber with no physical barrier between the meat and the surrounding environment (Stenström *et al.*, 2014). Within the hunting industry, it is also common practice to age carcasses skin-on(Jansen van Rensburg, 1997).

Game farming in South Africa is extensive in nature and the harvesting methods used will often span several days with the carcasses being kept in a refrigerated truck in the field (Bothma & Sartorius, 2016). In such a system, skin-on ageing is employed as an efficient way to allow all carcasses to be cooled together. Smaller scale operations are however able to reduce the period between harvesting, chilling and processing as the animals are typically culled in close proximity to a small-scale processing facility, thereby allowing them to extract the primal cuts and utilise vacuum bag ageing (VAC) on these cuts. Both methods have merits with regards to yield and turnover time. Logistically, skin-on ageing allows suppliers to carry out bulk processing after the entire harvesting process is completed (Radakovic & Fletcher, 2011). This reduces labour and transport costs in areas where processing plants are a long way from the farm. An added advantage to skin-on ageing is that the meat is only packaged once the ageing process has been completed whereas with vacuum ageing, meat is aged in a vacuum bag and might have to be repackaged once ageing is completed. Reduction in plastic use during production could appeal to environmentally conscious consumers. On the other hand, VAC ageing allows for faster turnovers in terms of cold room storage. Chiller space taken up by skin-on carcasses can only be utilised for new carcasses once the others have been removed in order to prevent overcrowding (FAO, 1991a; Department of Agriculture, 2000) thus limiting the capacity of the cold room when using skin-on ageing.

The moisture loss that occurs during skin-on ageing is another potentially negative factor. Moisture, the most dominant component of meat, evaporates from the carcasses and meat cuts when dry aged (Parrish *et al.*, 1991; Kim *et al.*, 2016). This moisture loss translates to a loss in carcass and muscle weight that impact on the earnings for the supplier. Additionally, trimming of dried surfaces of meat, particularly from cuts where excessive moisture loss has occurred, leads to further weight loss (Parrish *et al.*, 1991; Dikeman *et al.*, 2013) further affecting income generated. In skin-on ageing, the largest portion of the carcass is protected by the skin therefore moisture loss during this period would be far less compared to dry ageing (Jansen van Rensburg, 1997). Moisture loss during skin-on ageing likely occurs mostly in exposed muscles along the incision area (FAO, 1991a), most of which would be discarded in a commercial setting, as well as from the skin. There is also potential for meat contamination from the skin to the carcass surface during the skin-on ageing process as well as during subsequent skinning and this poses a safety hazard (Bell, 1997).

Studies on ageing meat have found that ageing method can also impact the type of microorganisms that grow during ageing (Newsome *et al.*, 1984; Nortjé & Shaw, 1989; Buys *et al.*, 1997; Hulánková *et al.*, 2018). Vacuum packaging is often used during wet ageing and has been shown to favour the growth of various Lactic Acid Bacteria (LAB) species more than an aerobic ageing environment would (Newsome *et al.*, 1984; Pothakos *et al.*, 2015), while *Pseudomonas spp.* is more dominant in aerobic environments (Nortjé & Shaw, 1989).

Ageing time also influences the type of microorganisms present during ageing. The decline in microbial diversity with time is as a result of processing and storage conditions exerting selective pressure on the microbial population present results in less microbial diversity at the end of storage (Zagorec & Champomier-Vergès, 2017). Additionally, the exponential growth of bacterial populations with time (Feiner, 2006) implies that longer ageing periods are more likely to produce meat with higher microbial counts (Newsome *et al.*, 1984; Nortjé & Shaw, 1989; Buys *et al.*, 1997; Hulánková *et al.*, 2018). Therefore, it is important to consider the effect of both ageing method and time on the overall quality of aged meat.

An optimal ageing period for springbok *Longissimus thoracis et lumborum* (LTL) muscles has been suggested as three to ten days (Jansen van Rensburg, 1997) or a maximum of eight days post mortem before sale (North, 2014). The aim of this study was to assess two ageing methods widely used on game meat in South Africa (skin-on and VAC) as well as the recommended springbok ageing periods (6, 7,8 and 9 days). The effect of ageing method and ageing time on the carcass attributes as well as physical attributes such as colour, pH, cooking loss, weep loss, Warner-Bratzler Shear Force (WBSF), microbiological and proximate composition of the springbok LTL muscles was determined.

3.3 Materials and methods

3.3.1 Harvesting, slaughter and ageing

Twenty-four springbok (twelve male and twelve female) were harvested from Brakkekuil farm in Witsand, Western Cape, South Africa according to standard operating procedures (SOP/ethical approval number SU-ACUM13-00034). The adult springbok were randomly harvested at night using a light calibre rifle fitted with a suppressor with a spotlight to immobilise the animals. A headshot was used to ensure instantaneous death. The carotid and jugular blood vessels were then severed to allow carcasses to bleed out while suspended from the transport vehicle before being transported to the onsite abattoir.

At the abattoir, all carcasses were suspended by both Achilles tendons and weighed. Thereafter, the heads and hoofs were removed and the carcasses, eviscerated within two hours post mortem. The carcasses were then suspended on hooks by both Achilles tendons in a cold truck and stored overnight at 0-4°C before transportation back to the meat science laboratory at the Department of Animal Science, Stellenbosch University the next day.

At the department, the carcasses were weighed and thereafter twelve (six males and six females) springbok were randomly selected for VAC ageing and the other twelve springbok were assigned to the skin-on ageing (six males and six females) for 6, 7, 8 or 9 days (Table 3.1). The former were skinned after selection and the LTL muscles excised (Fig. 3.1). The muscles were immediately weighed and vacuum sealed using a Multivac vacuum sealer (Model C200, Sepp Haggenmuller, Wolfertschwenden, Germany) in a vacuum bag with the following charateristics: 70 µm polyethylene and nylon; moisture vapour transfer rate of 2.2 g/m^2/24 h/1 atm, O_2 permeability of 30 cm^3/m^2/24 h/1 atm and a CO_2 permeability of 105 cm^3/m^2/24 h/1 atm. The VAC samples were then left to age for the time period allocated to each animal in a chiller at 2-4°C. At the end of the designated ageing period, the VAC samples were blotted dry, weighed and the epimysium removed. The skin-on carcasses were weighed and aged in the same cold room with a relative humidity of 79-93% for the allocated ageing time period. At the end of the ageing period, the skin-on carcasses were weighed, skinned, weighed again and the LTL muscles excised. These muscles were immediately weighed, vacuum-sealed and stored along with the VAC samples in the same cold room until physical analysis the next day.

Table 3.1 The number of animals randomly assigned to the treatments applied

Ageing time (T)	Skin-on ageing		VAC ageing		Total
	Male	**Female**	**Male**	**Female**	
Day 6	2	1	1	2	6
Day 7	1	1	2	2	6
Day 8	2	3	1	0	6
Day 9	1	1	2	2	6
Total	6	6	6	6	24

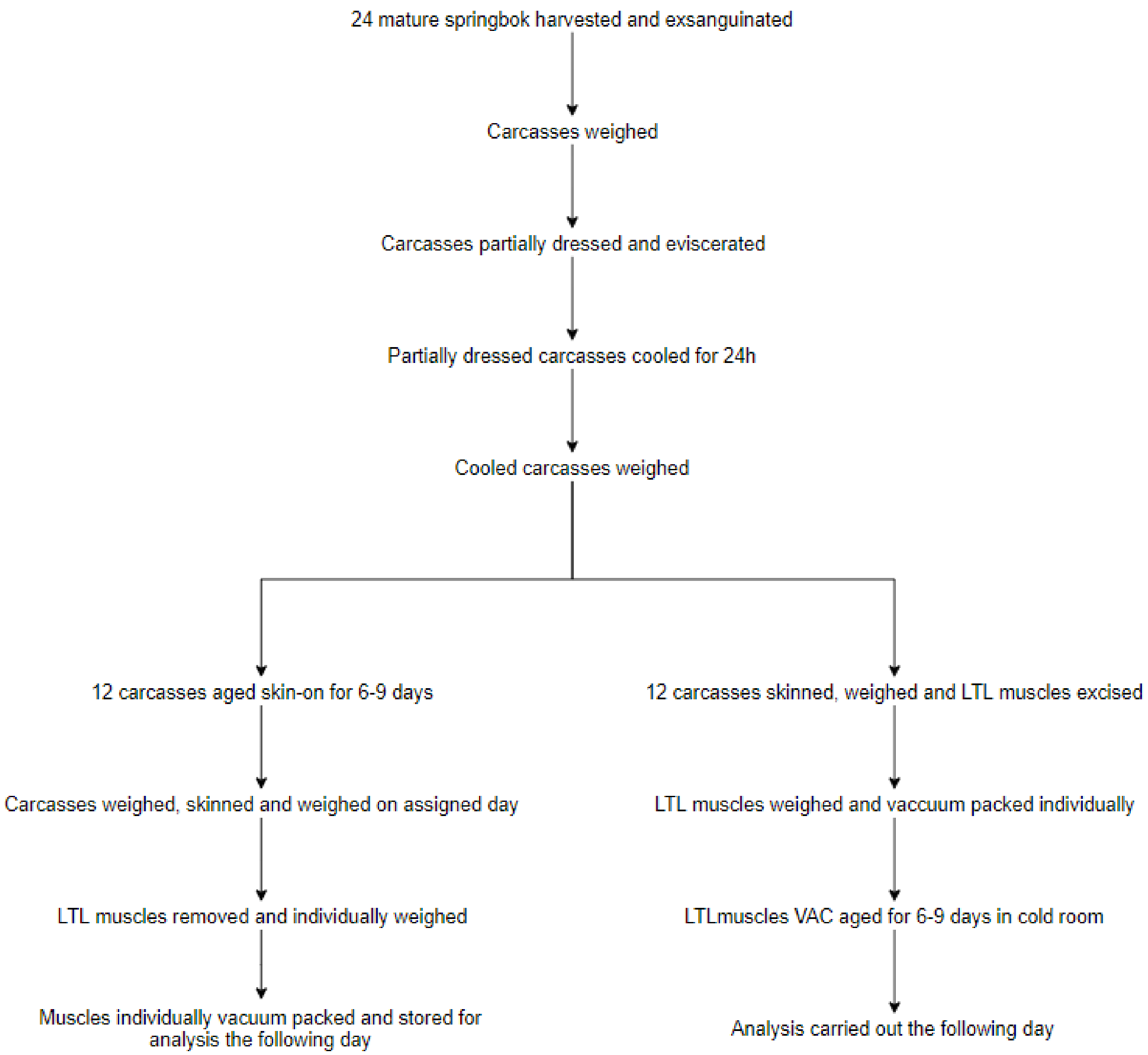

Figure 3.1 Process flow of carcass handling after harvesting has occurred up to end of ageing.

The different stages at which samples were obtained is illustrated in Figure 3.2. The pH_u, colour, proximate composition and weep loss were analysed on the right LTL while

WBSF, weep loss, cooking loss and microbial analysis were carried out on the left LTL muscle for all the animals. The epimysium of the skin-on samples was only removed after the weep loss weight was obtained.

3.3.2 Carcass weights

The dead weight was determined as the weight of the carcass after exsanguination. The dressing percentage was determined as a percentage of the weight of the carcass after skinning to the dead weight. For the skin-on aged carcasses, weighing was also done after the ageing period before and after skinning to determine percentage weight loss during the ageing period as well as the dressing percentage (using the skinned weight relative to the dead weight) (Fig. 3.1).

3.3.3 pH_u

The ultimate pH (pH_u) was determined at the end of each ageing period using a calibrated Crison pH25 pH meter (purchased from Lasec Pty Ltd, Cape Town, South Africa) fitted with a puncture electrode. The pH_u readings were taken from the *Longissimus thoracis* region of the LTL muscle (between 11th and 12th rib). The electrode was inserted at an angle into a fat free portion of the muscle and the pH_u and temperature readings taken.

3.3.4 Colour

A 2 cm thick steak was cut from the *Longissimus lumborum* portion from each LTL muscle perpendicular to the muscle grain. The steaks were then placed on a uniformly illuminated smooth surface and bloomed for 30 min at room temperature. The Color-guide 45°/0° colorimeter (BYK-Gardner GmbH, Gerestried, Germany) was used to take the colour measurements directly from the meat surface. Each sample was measured five times in different locations on the surface each time and the L*, a* and b* values were recorded. The chroma (C*) and hue angle were calculated using the formulas below (AMSA, 2012).

$$Chroma\ (C^*) = \sqrt{(a^*)^2 + (b^*)^2}$$

$$Hue\ (H_{ab}) = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

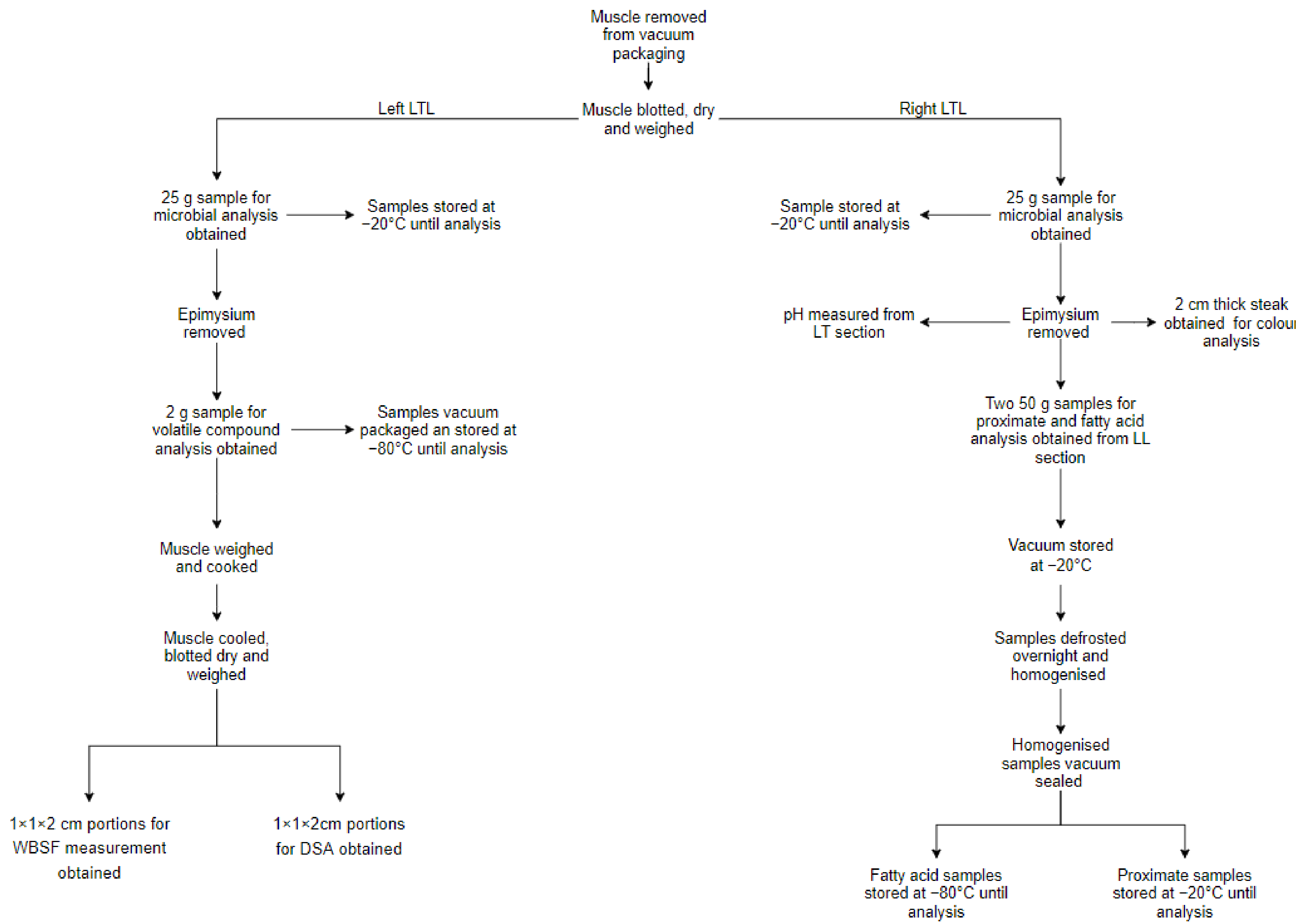

Figure 3.2 Layout for sample acquisition from muscles at the completion of ageing.

3.3.5 Weep loss

The VAC samples were weighed before the ageing period and after the ageing period had elapsed before removal of the epimysium. The muscles were blotted with an absorbent paper before being weighed. The percentage weep loss was then calculated using the equation:

$$\% \, weep \, loss = \frac{Weightof \, muscle \, before \, ageing - weight \, of \, muscle \, after \, ageing}{Weight \, of \, muscle \, before \, ageing} \times 100\%$$

The skin-on aged samples could only be weighed after the ageing period owing to the fact that they were still on the carcass. Once excised from the carcass at the end of their designated ageing time, the muscles were weighed and vacuum packed and stored in a 0-4°C chiller overnight. The muscles were then removed from the vacuum packaging, blotted dry with absorbent paper and then weighed. The weight was recorded and used to calculate weep loss using the following equation:

$$\% \, weep \, loss = \frac{Muscle \, weight \, before \, vacuum \, packaging - Muscle \, weight \, after \, vacuum \, packaging}{Muscle \, weight \, before \, vacuum \, packaging} \times 100\%$$

The average percentage for the left and right LTL muscle of each animal was recorded as the percentage weep loss.

3.3.6 Cooking loss

The percentage cooking loss was obtained from the samples used for Descriptive Sensory Analysis (DSA) (Chapter 5).

Meat samples were removed from the vacuum packaging and blotted with dry absorbent paper. The samples were weighed, the epimysium removed and then the samples weighed again. Each sample was then placed on a metal grid and then placed individually in labelled oven bags with a temperature probe inserted in the centre of the sample, fastened with a bag tie. The samples were placed in an industrial oven (Hobart, Paris, France) and cooked to an internal temperature of 72°C. Once cooking was complete, the samples were removed from the oven bag and allowed to cool for 5 min then blotted dry and weighed. The weight before and after cooking was recorded and used to calculate percent cooking loss using the following equation:

$$\% \, cooking \, loss = \frac{Weight \, of \, muscle \, before \, cooking - weight \, of \, muscle \, after \, cooking}{Weight \, of \, muscle \, before \, cooking} \times 100\%$$

3.3.7 Warner-Bratzler shear force (WBSF)

At least six 1 cm × 1 cm × 2 cm samples were obtained from both the *Longissimus lumborum* and *Longissimus thoracis* sections of each of the cooked LTL muscles that were used for Descriptive Sensory Analysis (DSA) (Chapter 5). The WBSF was measured according to Honikel (1998) using the Instron Universal Testing Machine (Instron UTM, Model 2519-107) fitted with a Warner-Bratzler blade in a room at 20°C. Cutting was done perpendicular to the direction of the fibre of the muscle and the shear force recorded in Newton (N).

3.3.8 Proximate analysis

A 50 g sample portion was obtained from the *Longissimus lumborum* portion of each animal's right LTL muscle after the ageing period had elapsed. The samples were homogenised (Dampa bowl cutter, CT, 35N), vacuum-sealed and stored at −20°C until proximate analysis was conducted. Samples were defrosted (0-4°C) overnight and analysed in duplicate.

Moisture analysis was according to AOAC official method 950.46 (AOAC, 2016a) and ash content determined according to AOAC official method 920.153 (AOAC, 2016b). Intramuscular fat (IMF) was extracted with a 1:2 chloroform/methanol solution as springbok meat has a fat content of < 5% (Hoffman *et al.*, 2007a; North & Hoffman, 2015; Neethling, 2016a) according to Lee *et al.* (1996). The defatted samples were used to determine protein content according to AOAC official method 992.15 (AOAC, 2016c) using the LECO FP528. The percentage nitrogen was used along with the conversion factor of 6.25 to obtain the percentage protein "as is" for the samples.

3.3.9 Microbial analysis

Two 25 g samples were obtained aseptically from the *Longissimus thoracis* portion of left LTL muscle of each animal. The samples were then vacuum sealed and frozen at -20°C until analysis. Sample preparation was according to AOAC official method 983.18 (AOAC, 2016d). The frozen samples were left to defrost overnight in a 0-4°C cold room. Each sample was then transferred to a stomacher bag along with 225 mL of 0.8% saline solution making a 10^{-1} dilution. The sample was then homogenised using a Stomacher (Interscience) for 2 min before a serial dilution was done up to 10^{-3}.

Microbial analysis for aerobic plate counts (APC), coliforms and *Escherichia coli* (*E. coli*) and lactic acid producing bacteria (LAB) was conducted using 3M petrifilms according to the specific AOAC methods (Table 3.2); 1 mL was taken from each dilution and plated on each petrifilm whilst a blank was also prepared by plating 1 mL of the unused diluent on a petrifilm. The petrifilm gels were allowed to solidify for 1 min before being incubated at the stipulated times and temperatures (Table 3.2). After incubation, colony forming units (CFUs) were enumerated by hand under light and then recorded as CFUs/g. The CFUs/g were converted to log CFUs/g and mean counts were obtained for each animal sampled.

Table 3.2 Methods, incubation time and temperature used for microbial testing.

Microorganism	Testing method	Incubation time	Incubation temperature
Aerobic plate counts	AOAC method 990.12	48h	35°C
Coliforms and *E.coli*	AOAC method 998.08	24-48h	35°C
Lactic acid bacteria	ISO 15214	48-72 h	30°C

3.3.10 Statistical analysis

Statistical analysis was carried out on the means of the parameters tested using statistical software, Statistica version 13.5. Mixed model analysis of variance (ANOVA) was conducted using the VEPAC module of Statistica 13.5 and the R "lmer" package. The animals were treated as random effect and sex, ageing method and time were fixed effects. For post hoc testing, Fisher Least Significant Difference (LSD) was conducted. Correlations between the different parameters were tested using XLStat (Version 2019.1.1.56421, Addinsoft, New York, USA) using the Pearson correlation function (r).

3.4 Results

Sex had no effect ($p = 0.639$) on dead weight of the springbok with a mean dead weight (± standard deviation) of 25.46 ± 3.32 kg reported (minimum = 17.6 kg and maximum = 32.1 kg). On average, the weight of the skin-on aged carcasses dropped by 5.17 ± 1.28% during the ageing period. Neither ageing time nor sex affected percentage weight lost during skin-on ageing (p = 0.343 and 0.853, respectively).

The mean dressing percentage across all treatments was 55.7 ± 4.04%. Skin-on samples had lower ($p = 0.047$) dressing percentage (54.5 ± 2.90%) than carcasses from which the VAC samples were extracted (56.8 ± 4.81%). There was no difference observed ($p = 0.102$) in dressing percentage with time post-mortem before skinning for skin-on aged carcasses (Fig. 3.3).

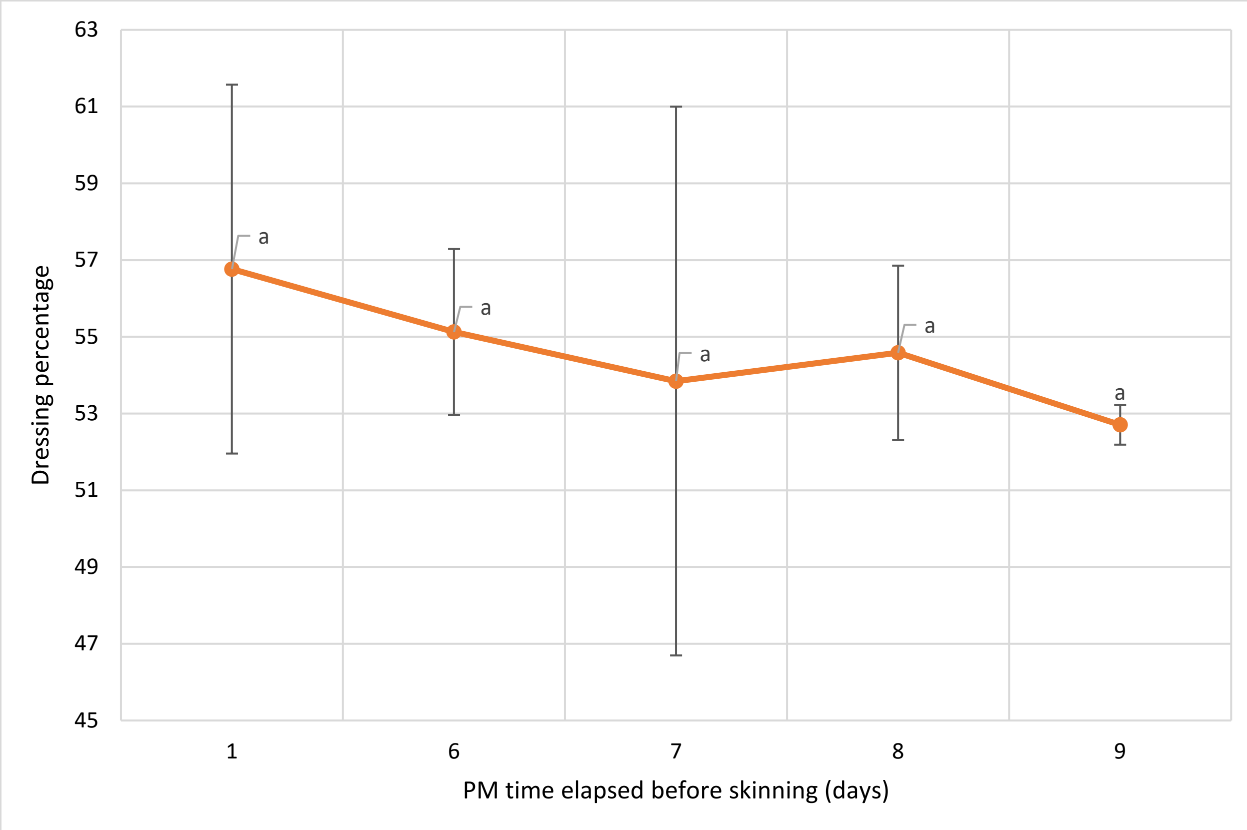

Figure 3.3 The change in mean dressing percentage of springbok carcasses with time post mortem (PM) ($p = 0.102$). Number of carcasses (n) varies on a given day (day 1 n = 12, day 6 n = 3, day 7 n = 2, day 8 n = 5 and day 9 n = 2). Error bars indicate standard deviation.

During analysis, samples from one springbok were classified as dark, firm and dry (DFD). This sample was therefore excluded during the statistical analysis of the physical and microbial properties and will be discussed separately. The effects of the main factors and the first order interactions on the physical and chemical attributes of the springbok meat is summarised in Table 3.3. There was no interaction between ageing method and sex (M*S) and sex and ageing time (S*T) for all parameters analysed while ageing method and time (M*T) only had significant interactions for a*, chroma and WBSF ($p \leq 0.050$) (Table 3.3); these will be discussed as such where appropriate. Sex (S) significantly influenced percentage moisture, IMF and cooking loss of the samples ($p < 0.050$). The ageing time (T) influenced the pH_u, b*, hue angle and cooking loss ($p < 0.05$) and had a slight effect on the aerobic plate counts (APC) ($p = 0.094$). The ageing method (M) only had an effect on percentage cooking loss ($p = 0.005$).

Table 3.3 The level of statistical significance (p-values) between main factors (ageing method, time and sex) and their interactions on physical, microbiological and chemical attributes.

	Ageing method (M)	Ageing time (T)	Sex (S)	M*T	M*S	S*T
Moisture (%)#	0.194	0.568	**0.016**	0.874	0.879	0.163
Protein (%)#	0.100	0.510	0.458	0.938	0.703	0.738
IMF (%)#	0.966	0.783	**0.004**	0.540	0.575	0.068
Ash (%)#	0.932	0.853	0.070	0.696	0.101	0.809
pH_u	0.117	**0.013**	0.538	0.376	0.733	0.504
L*	0.909	0.273	0.283	0.227	0.887	0.499
a*	0.247	0.317	0.068	**0.050**	0.516	0.124
b*	0.553	**0.028**	0.265	0.128	0.374	0.351
Chroma (C*)	0.282	0.139	0.080	**0.035**	0.921	0.119
Hue angle	0.855	**0.026**	0.772	0.581	0.306	0.811
Weep loss (%)	0.390	0.119	0.932	0.481	0.472	0.763
Cooking loss (%)	**0.005**	**0.022**	**0.044**	0.674	0.136	0.406
WBSF (N)	0.210	0.063	0.480	**0.040**	0.997	0.820
APC (log cfu/g)	0.955	**0.094**	0.833	0.510	0.723	0.347
LAB (log cfu/g)	0.102	0.305	0.758	0.545	0.918	0.629

For this attribute, sample characterised as DFD was included in statistical analysis

IMF Intramuscular fat

WBSF- Warner-Bratzler shear force

APC- Aerobic plate counts

LAB- Lactic acid bacteria

Female springbok had a lower moisture content than males (p = 0.016) while male springbok had a lower IMF content (p = 0.004). Ageing method and time did not affect the proximate composition of springbok meat (Table 3.4). The mean percentage proximate composition of aged springbok LTL muscles was 74.25 ± 1.50% moisture, 21.91 ± 0.76% protein, 2.37 ± 0.78% IMF and 1.24 ± 0.19% ash.

Ageing time had an effect (p = 0.013) on pH_u with day 7 having the lowest mean and pH_u thereafter increased significantly (Fig. 3.4). The mean pH_u recorded for all the samples was 5.45 ± 0.07.

The interaction between ageing method and time had a similar effect on both a* and chroma; day 6 skin-on aged samples had higher mean a* than day 6 VAC and day 8 samples (p = 0.050; Fig. 3.5a). The skin-on aged samples on day 6 also had higher chroma readings than VAC samples on the same day. Days 7 and 9 VAC aged samples had higher mean chroma readings than day 6 and day 8 VAC aged samples (p = 0.035; Fig 3.5b). Ageing time also had a significant effect on b* (p = 0.028) and hue angle (p = 0.026) with days 7 and 9 having higher b* readings than day 6 (Fig 3.6a). Days 7 and 9 also had the highest readings for b* and hue angle while day 6 had the lowest (Fig 3.6). The mean colour coordinates for aged springbok LTL muscles were 30.67 ± 1.50 for L*, 15.73 ± 1.55 for a*, 10.29 ± 1.841 for b*, 18.86 ± 1.82 for chroma and 33.06 ± 4.90 for hue angle.

The mean percent weep loss was 1.50 ± 1.33%; there was no significant effect of any of the main factors on weep loss (Table 3.3). Cooking loss was significantly affected by sex (p = 0.028), ageing method (p = 0.019) and ageing time (p = 0.022). Higher percentage cooking losses were reported for skin-on aged samples than VAC aged samples (Table 3.4). Females also had higher percentage cooking loss than males (Table 3.4). Day 6 had a lower cooking loss than day 7 with cooking loss declining with ageing time after day 7 (Fig. 3.7). The mean percent cooking loss varied from a minimum mean on day 6 of 27.6% to a maximum mean cooking loss of 31.5% on day 7.

The interaction between ageing method and time (M*T) had a significant impact on shear force (p = 0.040). Day 7 skin-on aged samples had significantly higher WBSF (64.17 ± 17.86 N) than the rest of the samples (Fig. 3.8) which is abnormally high for springbok meat. The mean WBSF for the springbok samples was 32.36 ± 15.79 N.

Only two samples tested positive for coliforms, consequently there was insufficient data to perform further statistical analysis. Additionally, none of the samples tested positive for *E. coli* indicating that there was no faecal contamination of the meat during the slaughter and handling process. The average plate counts recorded for both organisms were low (APC = 2.17 ± 0.52 log CFU/g and LAB = 0.49 ± 0.64 log CFU/g). None of the main factors had an effect on lactic acid bacteria (LAB) counts (p > 0.05) however, skin-on samples generally had lower (p = 0.102) LAB counts than VAC samples (Table 3.4). Additionally, of the 24 animals tested for LAB, only 10 animals tested positive. Seven of the 10 positive animals had undergone the VAC ageing (ca. 70% of positive samples and 58% of VAC samples). This would suggest that the VAC treatment promotes the growth of LAB during ageing. Ageing time slightly affected the APC (p = 0.094) with day 6 having lower counts than day 9 (Table 3.4).

Table 3.4 Means ± standard deviation of the main factors (ageing method, time and sex) on the physical, microbiological and chemical attributes of springbok *Longissimus thoracis et lumborum* muscle.

	Ageing method		Ageing time (days)				Sex	
	Skin-on	VAC	6	7	8	9	Male	Female
Moisture (%)[#]	74.6 ± 1.16	73.9 ± 1.74	73.6 ± 1.86	74.3 ± 1.94	74.6 ± 1.23	74.5 ± 1.37	75.0[a] ± 1.13	73.5[b] ± 1.48
Protein (%)[#]	21.6 ± 0.74	22.2 ± 0.69	22.3 ± 0.65	21.7 ± 0.75	21.6 ± 0.99	22.1 ± 0.55	22.1 ± 0.83	21.7 ± 0.67
IMF (%)[#]	2.4 ± 0.80	2.4 ± 0.80	2.4 ± 1.03	2.5 ± 1.06	2.4 ± 0.65	2.2 ± 0.35	1.9[y] ± 0.38	2.8[x] ± 0.82
Ash (%)[#]	1.2 ± 0.10	1.2 ± 0.25	1.2 ± 0.14	1.3 ± 0.33	1.2 ± 0.07	1.3 ± 0.14	1.2 ± 0.15	1.3 ± 0.19
pH_u	5.47 ± 0.05	5.43 ± 0.07	5.42[bc] ± 0.03	5.38[c] ± 0.06	5.49[ab] ± 0.06	5.50[a] ± 0.05	5.46 ± 0.07	5.44 ± 0.06
L*	30.50 ± 1.62	30.88 ± 1.32	31.23 ± 1.62	30.34 ± 1.65	30.03 ± 1.17	31.13 ± 1.29	30.41 ± 1.56	30.94 ± 1.40
a*	15.72 ± 1.57	15.74 ± 1.54	15.92 ± 1.74	15.94 ± 1.32	15.19 ± 1.79	15.94 ± 1.23	15.35 ± 1.70	16.11 ± 1.30
b*	10.07 ± 1.59	10.55 ± 2.09	8.79[b] ± 1.62	11.27[a] ± 1.79	10.06[ab] ± 1.75	10.94[a] ± 1.27	9.82 ± 2.02	10.76 ± 1.52
Chroma (C*)	18.73 ± 1.55	19.01 ± 2.11	18.21 ± 2.13	19.57 ± 1.69	18.30 ± 1.77	19.36 ± 1.36	18.30 ± 2.03	19.42 ± 1.38
Hue angle	32.65 ± 5.01	33.55 ± 4.76	28.73[b] ± 3.37	35.15[a] ± 4.27	33.54[ab] ± 5.68	34.45[a] ± 3.37	32.42 ± 5.39	33.71 ± 4.31
Weep loss (%)	1.08 ± 1.06	1.94 ± 1.46	1.03 ± 0.64	2.70 ± 2.09	0.99 ± 0.53	1.44 ± 1.02	1.37 ± 1.20	1.61 ± 1.45
Cooking loss (%)	31.17[x] ± 2.74	27.95[y] ± 3.27	27.62[b] ± 2.86	31.71[a] ± 4.02	31.16[ab] ± 1.93	28.38[ab] ± 3.34	28.32[b] ± 4.10	30.83[a] ± 2.03
WBSF (N)	33.79 ± 18.47	30.80 ± 12.11	30.11 ± 13.17	43.58 ± 22.37	27.08 ± 10.54	30.19 ± 10.57	33.91 ± 18.10	30.74 ± 12.84
APC (log cfu/g)	2.13 ± 0.68	2.21 ± 0.36	1.68[n] ± 0.57	2.13[mn] ± 0.44	2.35[mn] ± 0.35	2.51[m] ± 0.34	2.21 ± 0.30	2.13 ± 0.68
LAB (log cfu/g)	0.26 ± 0.39	0.74 ± 0.77	0.26 ± 0.60	0.34 ± 0.48	0.35 ± 0.58	0.99 ± 0.69	0.72 ± 0.84	0.41 ± 0.58

[a,b] Least square means in the same row (within the main factor) with different superscripts differ significantly from each other ($p<0.05$)

[m,n] Least square means in the same row (within the main factor) with different superscripts differ significantly from each other ($p<0.10$)

[x,y] Least square means in the same row (within the main factor) with different superscripts differ significantly from each other ($p<0.01$)

[#] For this attribute, sample characterised as DFD was included in statistical analysis

IMF Intramuscular fat

WBSF- Warner-Bratzler shear force

APC- Aerobic plate counts

LAB- Lactic acid bacteria

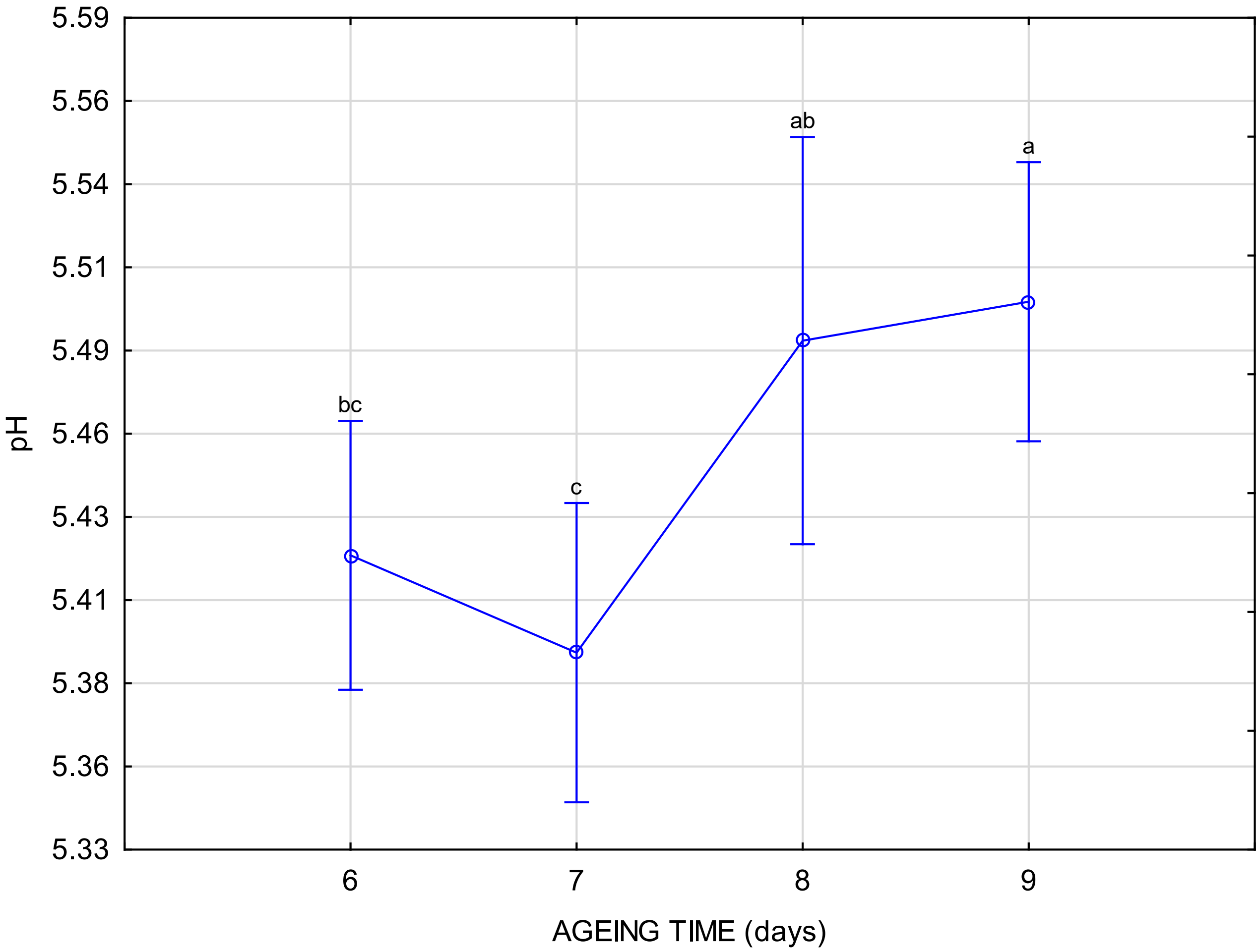

Figure 3.4 The changes in mean pH_u with ageing time for springbok *Longissimus thoracis et lumborum* muscle. [a,b] Means with different superscripts differ significantly ($p \leq 0.05$). Error bars indicate 95% confidence intervals.

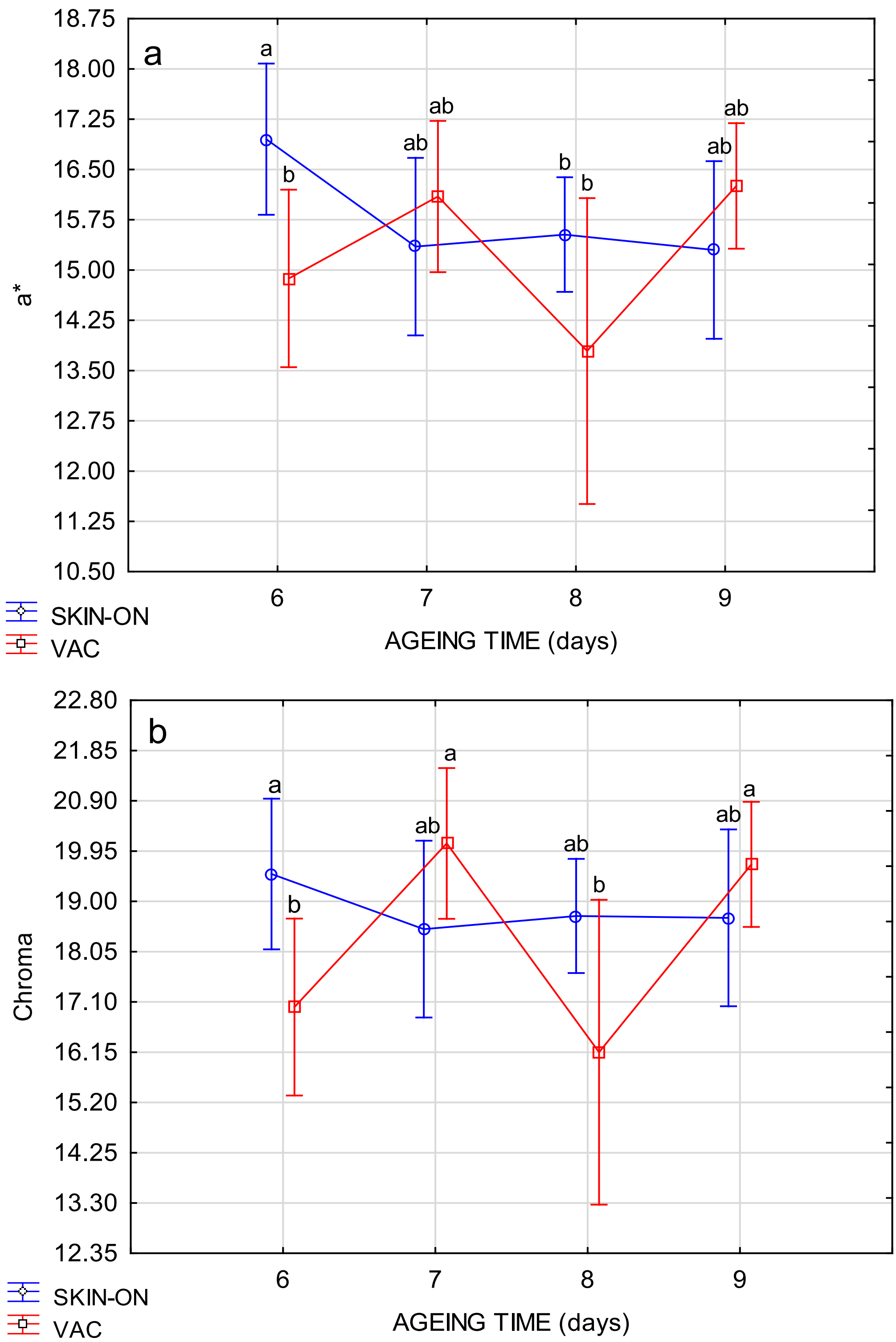

Figure 3.5 The changes in mean a* (a) and chroma (b) values with ageing time and ageing method for springbok *Longissimus thoracis et lumborum* muscle. [a,b] Means with different superscripts differ significantly ($p \leq 0.05$). Error bars indicate 95% confidence intervals.

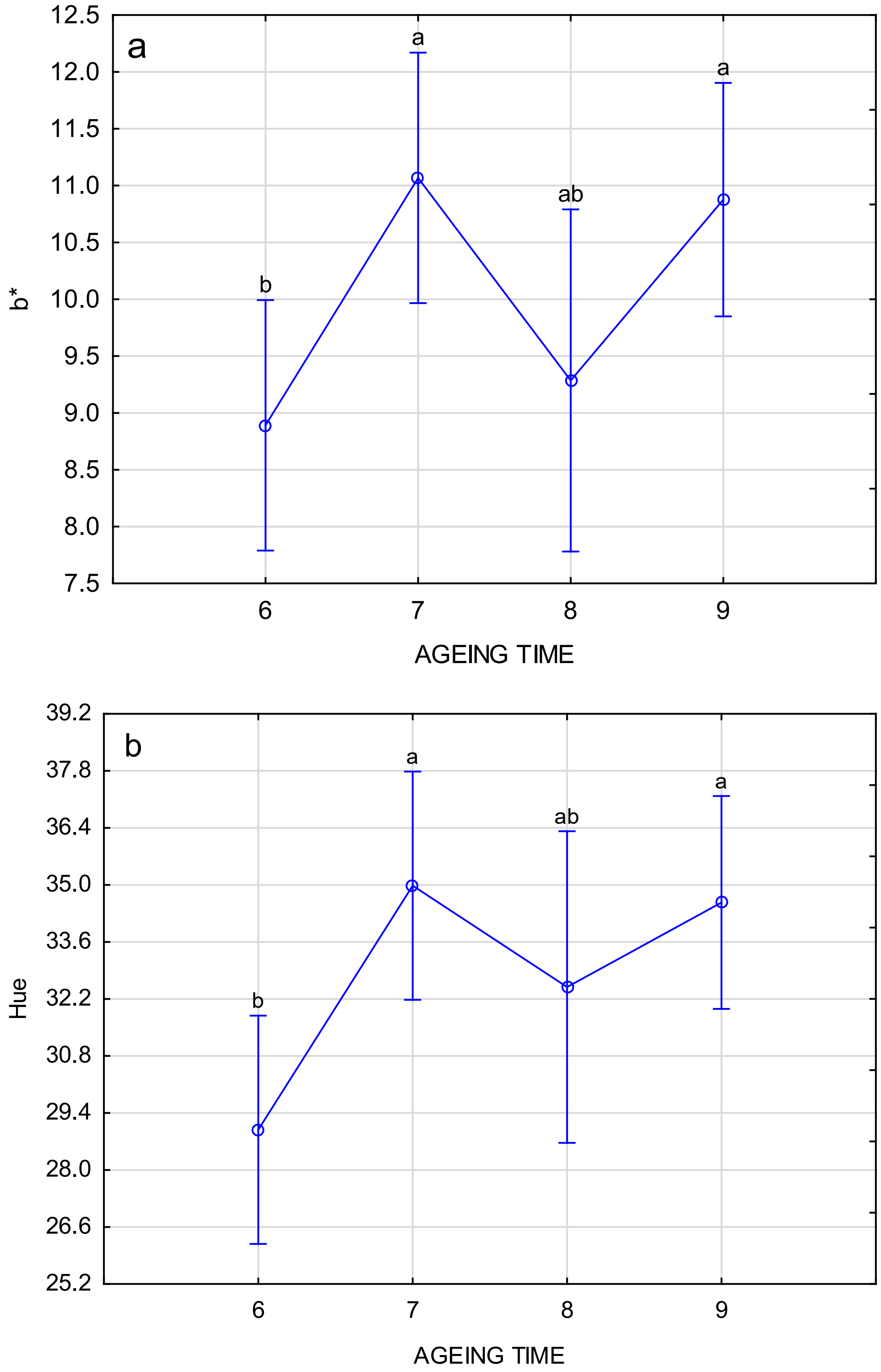

Figure 3.6 The changes in mean in b* (a) and hue angle (b) values with ageing time for springbok *Longissimus thoracis et lumborum* muscle. [a,b] Means with different superscripts differ significantly ($p \leq 0.05$). Error bars indicate 95% confidence intervals.

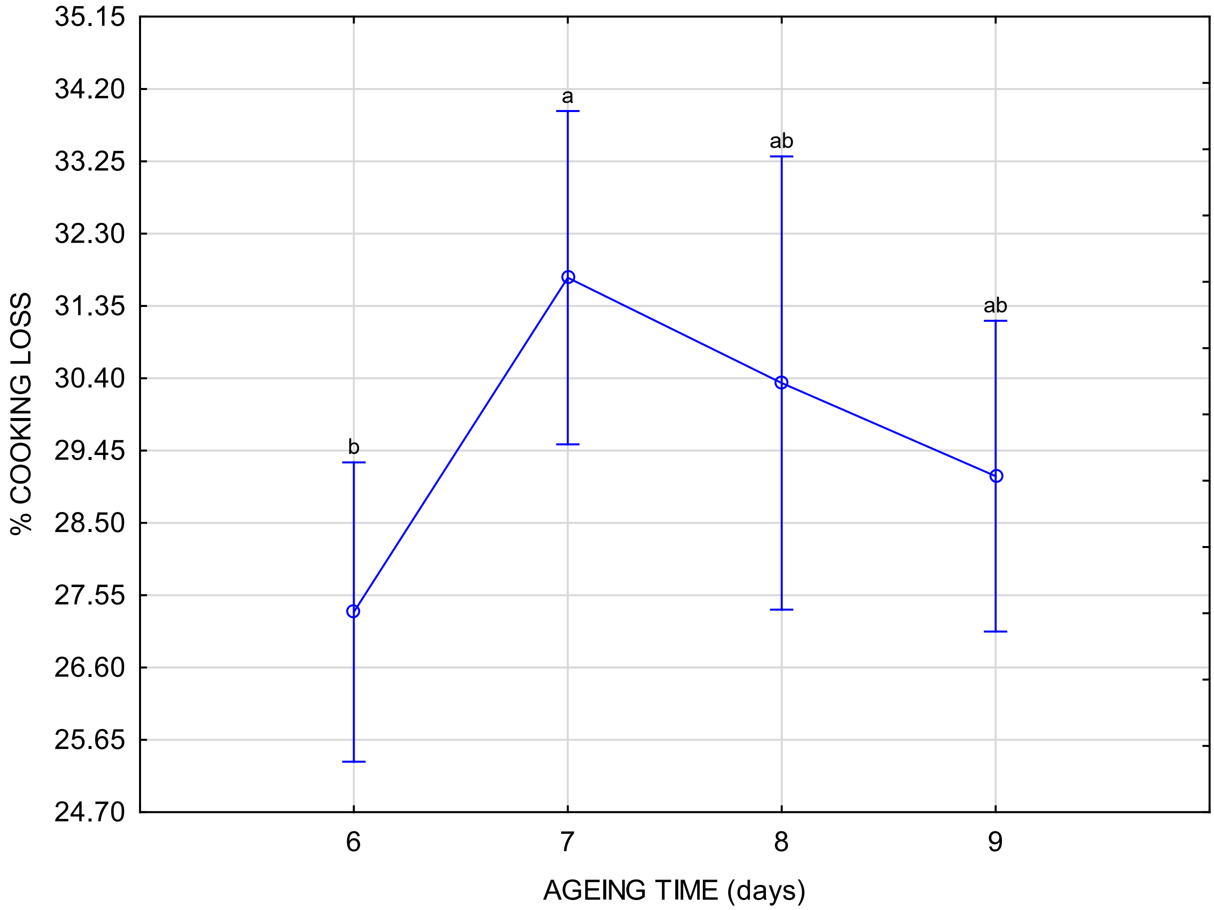

Figure 3.7 The changes in mean percentage cooking loss values with ageing time for springbok *Longissimus thoracis et lumborum* muscle. [a,b] Means with different superscripts differ significantly ($p \leq 0.05$). Error bars indicate 95% confidence intervals.

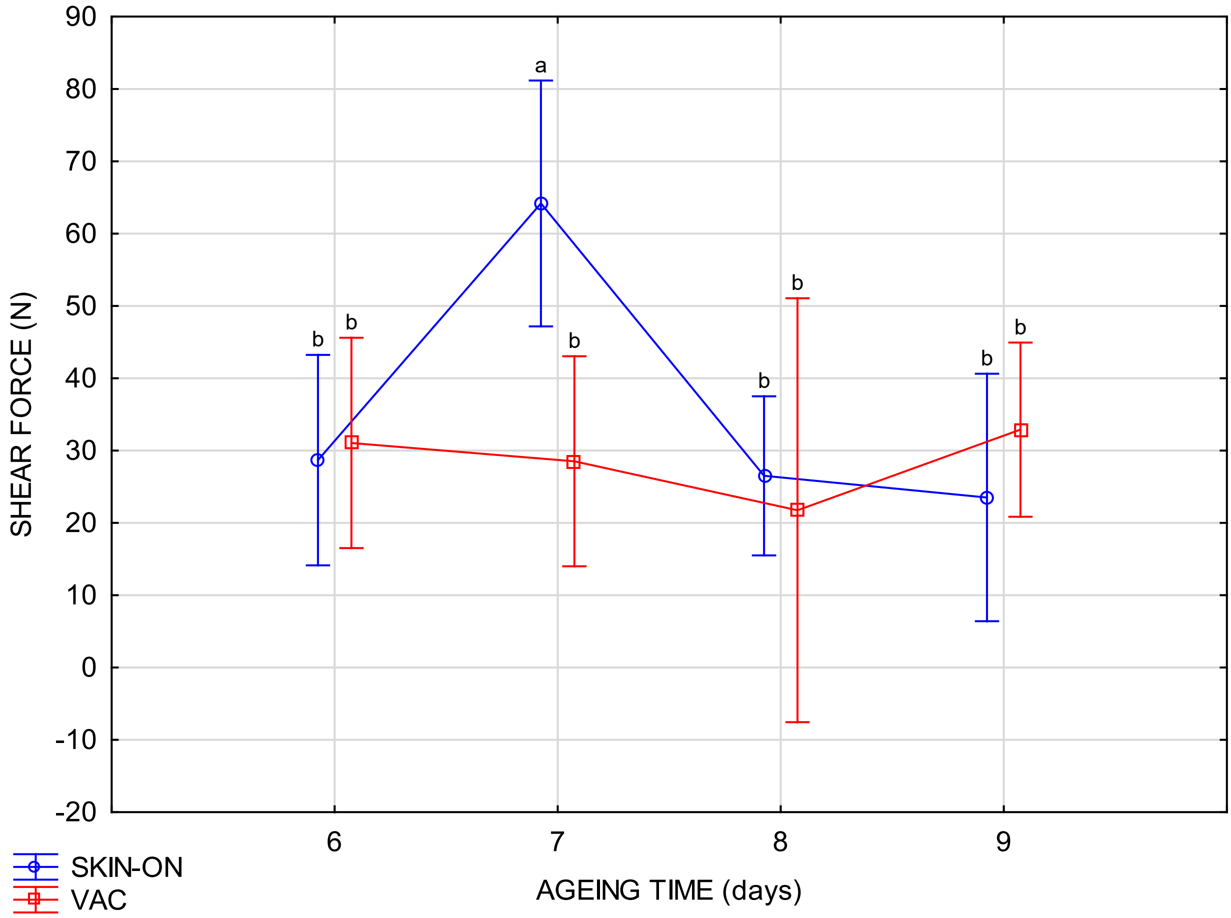

Figure 3.8 The changes in mean WBSF with ageing time (days) and ageing method for springbok *Longissimus thoracis et lumborum* muscle. [a,b] Means with different superscripts differ significantly ($p \leq 0.05$). Error bars indicate 95% confidence intervals.

The DFD male springbok was characterised as such due to its high pH_u of 6.49. The mean proximate composition of this sample was 77.16 ± 0.19% moisture content, 21.04 ± 0.08% protein, 1.46 ± 0.02% IMF and 0.77 ± 0.17% ash. The colour readings for the sample were 26.43 ± 1.41 L*, 9.76 ± 1.61 a*, 6.16 ± 1.60 b*, 11.62 ± 1.25 chroma and 32.08 ± 7.65 hue angle. The cooking and weep losses of the sample were 18.53% and 0.33% respectively. The mean WBSF recorded for the sample was 42.60 ± 7.51 N that was higher than the mean WBSF recorded for the rest of the samples (Fig. 3.9). Mean APC of 2.89 log CFU/g and LAB count of 2.28 log CFU/g were also recorded for this sample.

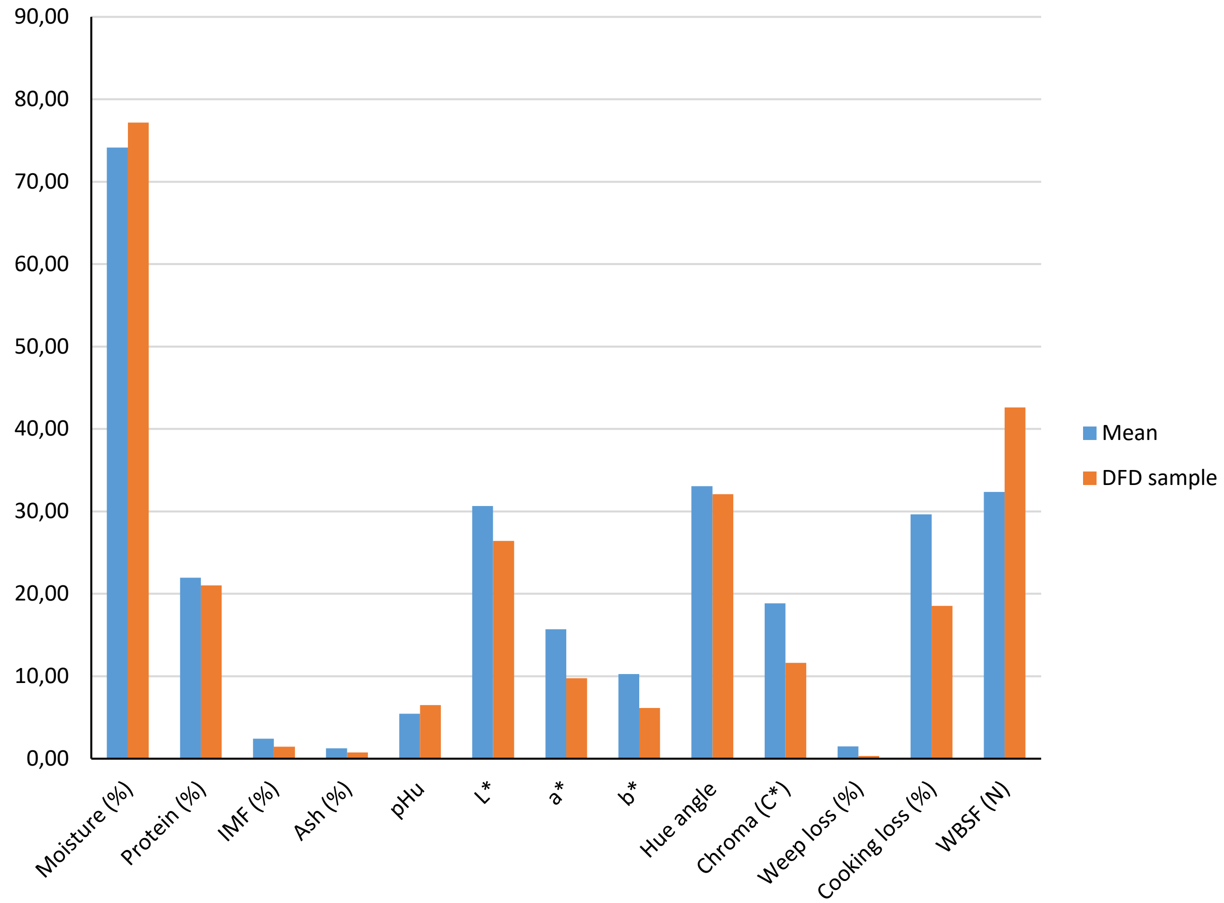

Figure 3.9 Comparison of the chemical and physical attributes between the DFD and the mean values across all ageing treatments for 'normal' meat for springbok *Longissimus thoracis et lumborum* muscles.

3.5 Discussion

The mean dead weight reported in this study is in line with dead weights previously reported for sub-adult springbok from the same region (24.68 kg ± 0.51) (Kroucamp, 2004). The dressing percentage reported was also in line with the dressing percentages of springbok from various regions of South Africa (Kroucamp, 2004; van Zyl & Ferreira, 2004; Neethling, 2016a). The change of dressing percentage with time post-mortem (Fig. 3.3) suggests that the overall weight loss during ageing is not affected by ageing method or time used. As carcass weight translates into income generated (ZAR/kg), significant differences in carcass weight would translate in differences in income generated.

As the weight lost in the skin-on carcasses was unaffected by ageing time, the bulk of moisture lost during the skin-on ageing therefore occurred within the first six days of ageing. The recorded weight loss is likely due to moisture loss from the exposed surfaces on the carcasses, particularly from the skin surface (as the carcasses had all been washed after evisceration and before being placed into the chiller) and within the carcass through the mid-ventral slit made during the evisceration. Additionally, the muscles along the opened mid ventral region were much darker and dryer than observed with the rest of the muscles once the carcass had been skinned. The region resembled the characteristic dried layer formed on the surface of meat during dry ageing. These exposed muscles would likely have to been trimmed off the carcass and disposed of as trimmings (Parrish *et al.*, 1991; Campbell *et al.*, 2001). The weight of these trimmings was not recorded in this study as it was not deemed to be significant enough to affect the overall percentage yield; these abdominal muscles of springbok carcasses are very thin, have no subcutaneous fat and are nearly always discarded in commercial plants as they have a high level of collagen and are deemed to be unsuitable for further processing (Van Schalkwyk & Hoffman, 2016).

As with previous studies in aged meat, few to no significant changes to moisture, protein and ash content (Table 3.4) were observed as a result of ageing (Parrish *et al.*, 1991; North & Hoffman, 2015; Soriano *et al.*, 2016). Previous studies on both fresh and aged springbok meat show a similar trend with regards to IMF content and sex (Table 3.4) with females suggested to have inherently more IMF deposited than males (van Zyl & Ferreira, 2004; Hoffman *et al.*, 2007a; North, 2014; Neethling *et al.*, 2018). A strong negative correlation between the moisture and IMF content was observed ($r = -0.814$; $p < 0.0001$) indicating that a higher IMF content would result in lower moisture content and would explain why these two parameters differed significantly between meat from male and female springbok (Table 3.4).

The ageing method did not affect the IMF content of springbok LTL muscles in the current study ($p = 0.966$). Dikeman *et al.* (2013) found that dry aged steaks had higher percentage fat than VAC aged and special bag aged beef steaks ($p = 0.04$); likely due to greater moisture loss in dry aged samples than in the other two treatments. However, since the skin-on ageing used in the current trial more closely mirrors the special bag and VAC ageing techniques than dry ageing, a better comparison would be between the VAC and special bag aged samples in that study; fat content did not differ significantly.

The mean pH_u recorded in this study is similar to previous studies on aged springbok meat (North & Hoffman, 2015) where no difference in pH_u with ageing time was reported ($p = 0.952$). Decrease in pH during aerobic storage with time was observed in black wildebeest (*Connochaetes gnou*) until day 9 where after there was an increase in pH from day 9 to day 12. Additionally, there was an overall significant increase ($p \leq 0.05$) in pH from day 0 to day 12 (Shange *et al.*, 2019). Increase in pH during storage and ageing has been reported previously and attributed to the increase in ammonia and other basic compounds produced as a result of bacterial activity during storage (Rodríguez-Calleja *et al.*, 2005; Lawrie & Ledward, 2006; Neethling *et al.*, 2019); this could be the cause of the slight increase in pH_u reported in this study (Fig. 3.4).

The lower hue angle recorded in day 6 samples shows that these samples are significantly more to the red side of the scale than the yellow (Setser, 1984; AMSA, 2012). An increase in hue angle has been shown to indicate an increase in discolouration of meat (Shange *et al.*, 2019). The use of hue angle as a measure of discolouration is further backed by a significant strong correlation between hue angle and percentage metmyoglobin (% MMb) values ($r = 0.83$, $p \leq 0.05$) in springbok meat (Neethling, 2016b). This would therefore suggest that the colour of samples after 6 days of ageing was more desirable than the samples aged for longer. Additionally, the higher a* and chroma of day 6 skin-on aged samples suggests that they would appear redder than the VAC samples aged for the same amount of time. A significant positive correlation between a* and chroma ($r = 0.94$, $p \leq 0.05$) and percentage oxymyoglobin (% OMb) has been established in springbok meat (Neethling, 2016b). The positive correlation between a* and chroma was reported in this study ($r = 0.89$, $p < 0.0001$) and is further emphasized by the similarity in the trends of the two attributes (Fig. 3.5). In general, the skin on samples showed a more consistent trend for both a* and chroma readings as ageing time progressed than the VAC samples (Fig. 3.5). In red deer (*Cervus elaphus*), a* and chroma values highly positively correlated to the colour perceived by the trained panel (Stevenson *et al.*, 1989). Relating these findings to the current study, it would therefore suggest that on day 6, skin-on aged samples would have a more desirable colour than the VAC samples.

Unlike the current study, a previous study (North & Hoffman, 2015) on aged springbok meat found no effect of ageing time on cooking loss ($p = 0.132$). However, this was attributed to the greater freeze-thaw losses that occurred prior to cooking (North & Hoffman, 2015). The highest cooking loss on day 7 could be linked to the pH_u on this day, which was the lowest of all the readings on the specific days (Figs. 3.4 & 3.7). Furthermore, weep loss was noticeably higher on day 7 than any of the other ageing days (Table 3.4). Literature suggests that an increase in moisture losses can be expected as the pH of meat drops towards its isoelectric point of 5.4-5.5 due to the denaturation of muscle fibres as well as the shrinkage of intercellular spaces (Matarneh *et al.*, 2017; Warner, 2017). However, day 6 samples which had the lowest cooking loss still had a pH_u within the isoelectric point range and did not differ from pH_u on day 7 ($p > 0.05$). Additionally, pH_u on day 6 was significantly lower than that on day 9 (Fig. 3.4) but the cooking losses between these two days did not differ significantly (Fig 4.7). Purchas (1990) identified a curvilinear relationship between cooking loss and pH_u ranging from 5.4 − 7.0 in beef indicating that the relationship between pH_u and cooking loss is not always linear. The linear correlations established between pH_u and cooking and weep losses in the current study were weak ($r = 0.126$; $p = 0.568$ and $r = -0.439$; $p = 0.036$ respectively). This suggests that either pH_u was not the only factor affecting cooking loss or that as with beef, the relationship between pH_u (Table 3.3) and cooking loss in springbok is not strictly linear.

Ageing method and sex both had reported significant differences in cooking loss but none with pH_u further suggesting that pH_u alone was not the single determinant of cooking loss. Previous studies on cooking loss showed that presence of less moisture as a result of dry ageing resulted in lower cooking losses in dry aged meat than wet aged meat (Oh *et al.*, 2018). Lower cooking loss in VAC samples was coupled with higher weep loss and lower pH_u compared to skin-on samples; the cumulative influence of which could have resulted in lower cooking losses (Table 3.4). However, female springbok had a higher mean cooking loss, slightly lower weep loss and pH_u as well as significantly lower moisture content (Table 3.4).

Meat from male springbok has previously exhibited higher cooking losses than meat from female springbok (Hoffman *et al.*, 2007b; North & Hoffman, 2015) ($p < 0.05$ and $p = 0.016$, respectively) which is in contrast to the findings of this current study (Table 3.4). However, in the study by Hoffman *et.al.* (2007b), the males also had significantly lower pH 24h post-mortem (pH_{24}) than females ($p < 0.05$) and a weak negative correlation between pH_{24} and cooking loss ($r = -0.42$ $p < 0.001$). It is therefore likely that the difference in cooking loss with sex was partially influenced by pH_u.

It is important to bear in mind that in the current study, assessing how intrinsic differences between the different ageing methods and time periods affected subsequent moisture loss is difficult as samples from different ageing methods and time periods were obtained from different carcasses i.e. each animal was subjected to one ageing time point and one ageing method. To better understand how the differences in intrinsic parameters e.g. pH_u change during ageing and thus affect moisture loss, it is recommended that a single carcass be subjected to different ageing times although the practicality of this with skin-on ageing is questionable. However, from the current findings 6 day VAC ageing would be recommended as it yielded the least cooking loss.

There were no significant effects of any of the factors on weep loss (Table 3.3). This was unexpected as the weep loss recorded for the VAC samples was over the entire ageing period whereas the weep loss in the skin-on samples was only for an overnight vacuum storage period (Fig. 3.1). The LTL muscles that underwent skin-on ageing could only be excised after the ageing period had elapsed. Perhaps a better comparison for weight loss between the two ageing period would be in comparing the weight of loins to that of the cold carcass. This would take into account the price paid for the cold carcass (ZAR/kg) compared to the price that can be paid for the LTL muscle after ageing. In that respect, it would appear that in some cases there is a greater decrease in LTL muscle weights in relation to the cold carcass weight than in others (Addendum A). Further analysis of the effect of ageing on the eventual yield and income can be elucidated upon in future studies.

In the analysis of parameters pertaining to moisture and the loss thereof (moisture content, weep loss, cooking loss and weight loss during ageing), the only parameter that was found to differ significantly between ageing methods was cooking loss (Table 3.4). Although, VAC ageing resulted in a lower cooking loss than skin-on ageing ($p = 0.019$), the skin-on samples had higher moisture content ($p = 0.194$) and lower weep loss ($p = 0.390$). Similarly, ageing time used was found to only affect cooking loss ($p = 0.022$). Based on this, ageing for 6 days would yield the least moisture loss and therefore weight loss. Further investigation on the impact of these findings on the overall sensory quality of meat will be discussed later in Chapter 5.

The day 7 skin-on samples had significantly higher WBSF (64.17 ± 17.86 N) than all the other samples (Fig. 3.8). These shear force values were considered abnormal as they were far above those previously reported in springbok meat (Jansen van Rensburg, 1997; Hoffman *et al.*, 2007b; North & Hoffman, 2015; Neethling *et al.*, 2018). Although pH_u is a major factor influencing shear force (Purchas *et al.*, 1999; Hopkins, 2017), these two animals had normal pH_u values (5.44 and 5.45). As there appears to be no trend of this effect of ageing method and time on WBSF, it is likely that the reported values occurred as a result of inter-animal variation such as age or ante mortem stress levels. If the two animals were considered as outliers, the mean WBSF values for day 7 would be 29.26 ± 11.16 N that are in line with WBSF readings from the rest of the ageing days (Table 3.4).

The mean APC and *E. coli* and coliform counts (2.17 ± 0.52 and < 1.00 log cfu/g, respectively) observed for the skin-on aged samples were far lower than those anticipated when indirect contamination of carcasses from the skins has occurred. In the instance of indirect carcass contamination, Bell (1997) reported 5.00 log cfu/cm^2 and 2.40 log cfu/cm^2 for APC and *E. coli* respectively on beef carcasses. Additionally, none of the samples in the current study tested positive for *E. coli* indicating that there was no faecal contamination of the meat during the slaughter and handling process. Low microbial counts observed in skin-on aged LTL muscles in the current study were expected as during the skin-on ageing period, the LTL muscle does not come in contact with the outer skin of the carcass. Furthermore, this illustrates that subsequent skinning and muscle excision after ageing did not result in higher numbers of APC than with the VAC method. The lack of difference between mean APC for the two ageing methods (Table 3.4) shows that skin-on ageing and subsequent skinning can proceed without indirect contamination of the LTL muscle with microorganisms present on the skin. It is also possible that moisture loss from the skin resulted in lower microbial load on the skin thereby also reducing the potential for skin to carcass contamination during skinning. However, this cannot be definitively proven in the current study as no microbial testing was done on the skins. It can therefore be expected that majority, if not all of the primal muscles would not be cross contaminated as they too do not come in contact with the skin during the ageing period. The only place where indirect carcass contamination can occur is around the belly incision made during the evisceration and/or the region where the bung is removed (Van

Schalkwyk & Hoffman, 2016). However, as discussed earlier, these belly muscles are typically removed and not processed further.

The trend for increasing APC with ageing time (Table 3.4) has previously been reported (Newsome *et al.*, 1984; Nortjé & Shaw, 1989; Buys *et al.*, 1997). The mean APC throughout the trial ranged from 1.7 to 2.5 log CFU/g; below the 3.5 log CFU/cm^2 lower limit set for human consumption according to EU regulation No.2073/2005. The inverse relationship observed between initial bacterial load at the start of storage and subsequent shelf-life (Nortjé & Shaw, 1989; FAO, 1991b; Samelis, 2006) suggests that the specific samples that were aged for 6 days in the current trial would have a longer shelf-life on retail than samples aged for 9 days.

Although the mean LAB count was low for all samples, LAB counts were higher in the VAC aged samples ($p = 0.102$; Table 3.4) than in the skin-on aged samples. Additionally, a higher percentage of samples with LAB present underwent VAC ageing suggesting that VAC treatment promotes the growth of LAB. This is consistent with other studies on beef and springbok (Newsome *et al.*, 1984; Buys *et al.*, 1997). The anaerobic environment created due to vacuum packaging facilitates greater growth of LAB (Pothakos *et al.*, 2015). LAB growth can result in acidification of meat and production of off-flavours compounds in meat resulting in spoilage (Jones, 2004; Schillinger *et al.*, 2006; FAO, 2013; Pothakos *et al.*, 2015). In addition to causing spoilage, LAB have also been suggested to influence the acidic or sour aroma and flavour reported in aged meat (Lawrie & Ledward, 2006; Iulietto *et al.*, 2015) however, the mean counts found in this study were far below those at which sour flavour and aroma would to be detected (Korkeala *et al.*, 1990; Kalschne *et al.*, 2015).

The difference between LAB counts from skin-on and VAC ageing could become impactful during storage for retail. In beef steaks aged for three weeks and then packaged for retail in oxygen-permeable fresh meat cellophane, steaks that had been conventionally dry aged had consistently lower LAB counts than those that had been vacuum aged ($p < 0.01$) on corresponding storage days (Newsome *et al.*, 1984) while all other microbial counts did not differ ($p \geq 0.05$). This is likely due to lower initial LAB counts present at the start of storage in the dry aged samples; as with skin-on aged samples in the current study.

The DFD sample in this trial was a good illustration of the relationship between pH_u and moisture retention in meat (Lawrie & Ledward, 2006; Warner, 2017) as well as its effect on colour and microbial quality of game meat (Shange *et al.*, 2019). The male 7 day VAC aged sample had a cooking loss of 18.53% and weep loss of 0.33%; far lower than the overall average cooking and weep losses than the rest of the samples (Fig. 3.9) and higher mean APC and LAB counts (2.89 log CFU/g and 2.28 log CFU/g, respectively) than the rest of the samples. Game meat classified as DFD have been shown to exhibit this trend of higher (p <

0.05) water-holding capacity and lower cooking and drip losses (Hoffman *et al.*, 2007b) as well as higher microbial counts (Shange *et al.*, 2019).

Shorter shelf-life has been reported in DFD meat in black wildebeest (*Connochaetes gnou*) samples (pH > 6.06) stored in aerobic conditions, a shorter shelf-life was reported as a result of more rapid APC and *Enterobacteriaceae* growth (Shange *et al.*, 2019). The high pH samples reached the microbial spoilage limit four days earlier than the normal pH samples. Similarly, LAB growth in three week VAC aged beef steaks resulted in spoilage after 14 days of modified atmosphere package storage while one week aged steaks only spoiled after 21 days (Nortjé & Shaw, 1989). The significant decrease in shelf-life is a characteristic generally associated with high pH in meat (Wiklund *et al.*, 1995). The higher APC and LAB counts found for the DFD sample in the current study suggest that a similarly shorter shelf-life can be expected for DFD samples in springbok.

The DFD sample had the lowest L*, a*, b*, chroma and the highest moisture content (Fig. 3.9), appeared visibly darker than the rest of the samples and had a sticky texture. The colour coordinates for this sample also fell within the range suggested by Shange *et al*. (2019) for classification as DFD in game meat (L* < 30, a* < 11, b* < 7, chroma < 13 and hue angle < 32). The high pH_u in this particular sample is likely due to stress during harvesting that resulted in rapid ante-mortem glycogen depletion. Previous studies have suggested stress during harvesting results in a high pH_u as stress leads to rapid ante-mortem glycogen depletion resulting in insufficient pH drop post-mortem (Wiklund *et al.*, 1995; Hoffman *et al.*, 2007b). Field notes taken during this harvesting period suggest that this may have been the case as this animal was among the last in a group that was culled. This animal was also relatively smaller than the other animals harvested in this group and would likely experience glycogen depletion faster under similar stressful conditions.

3.6 Conclusion

Skin-on aged carcasses had a lower dressing percentage than carcasses skinned after 24 h due to moisture loss from skin and muscle surfaces exposed during the ageing period in the chiller. However, this weight loss was attributed to the washing of carcasses and slow drying of residual water on the external side of the skin. In terms of appearance, the stable trends observed in colour coordinates with ageing time for skin-on aged samples suggest that skin-on ageing results in more predictable colour coordinates than VAC ageing when ageing time is varied; on day 6, skin-on ageing was also found to produce meat with a more desirable colour than VAC ageing. The source of variation in cooking loss between the different treatments should be examined further in order to confirm findings in the current study. It would however appear that ageing male springbok 6 days in vacuum packaging would yield the lowest cooking loss. The effect of pH_u on microbial activity, colour and moisture related attributes in springbok was also illustrated with a high pH_u resulting in DFD meat with higher moisture content and microbial counts and lower cooking and weep loss.

Overall, few differences in the attributes examined arose as a result of the different ageing methods and ageing times applied. Nonetheless, from these findings 6-day skin-on ageing would result in more desirable colour for springbok meat as well as lower APC potentially resulting in longer shelf-life. This study also shows that the previously recommended ageing times (ca. 6 days) for springbok are capable of producing meat of acceptable microbial quality as per the EU regulation No.2073/2005 provided good operating procedures are adhered to. Further investigation into how ageing method and time affect shelf-life of springbok can be undertaken to determine retail time once ageing is complete.

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CHAPTER 4

The effects of ageing method and time on fatty acids and volatile compound profile of springbok (*Antidorcas marsupialis*) *longissimus thoracis et lumborum* muscle

4.1 Abstract

This study aimed to establish the fatty acid and volatile compound profiles of aged *Longissimus thoracis et lumborum* muscles of 24 mature springbok (12 males and 12 females), as well as determining what effect the ageing method [skin-on and vacuum bag ageing (VAC)] and ageing time (6, 7, 8 and 9 days) applied have on the aforementioned profiles. Saturated fatty acids (SFA) were the most abundant fatty acids irrespective of treatment applied followed by polyunsaturated fatty acids (PUFA) and then monounsaturated fatty acids (MUFA). The mean PUFA to SFA ratios (PUFA:SFA) and omega-6 to omega-3 (n-6:n-3) PUFA ratios were within the recommended limits at 0.65 ± 0.42 and 0.58 ± 0.15, respectively. Interactions between treatments were reported for dihomo-γ-linolenic acid (ageing method*time; $p = 0.021$), total n-3 (ageing method*sex: $p = 0.045$), eicosadienoic acid and arachidonic acid (ageing time*sex; $p = 0.035$ and $p = 0.019$, respectively). Ageing method alone did not impact the fatty acid profile of springbok meat while several fatty acids were affected by sex. Fifty-three volatile compounds were tentatively identified in aged springbok; majority of which were alcohols and esters. The most abundant volatile compound identified was acetoin which was higher in VAC aged samples ($p = 0.048$) and the meat derived from females ($p = 0.043$). Aged springbok meat also contained noticeably more volatile compounds that were previously undetected in fresh springbok meat.

Keywords; Skin-on ageing, game meat, n 6:n-3 PUFA ratios, nutritional value of springbok meat, PUFA:SFA ratio

4.2 Introduction

The fatty acid profile is an important quality parameter as it gives an indication of the nutritional value of meat (WHO, 2003; Astrup *et al.*, 2008). Humans are able to synthesize majority of the fatty acids required by the body with an exception of two essential fatty acids; linoleic acid (C18:2n-6c) and α-linolenic acid (C18:3n-3). The inability of the human body to synthesize these fatty acids as well as their function as substrates for synthesis of longer chain fatty acids means that these essential fatty acids must be obtained from dietary sources (Sprecher, 1992; Whitney & Rolfes, 1999; Yehuda, 2009). Meat is a good source of these essential fatty acids as well as other long chain unsaturated fatty acids (Hoffman *et al.*, 2007; Murphy & Howe, 2009; Neethling *et al.*, 2018).

Meat however is also rich in saturated fatty acids (SFA); high intake of which has been linked to increased risk of cardiovascular disease (Anderson & Ma, 2009; Schmid, 2011). Generally, decrease, not elimination, in SFA intake is recommended for a healthy diet (EFSA, 2010; Wood, 2017). Linoleic acid intake in the modern diet has increased due to increased use of linoleic acid rich vegetable oils and feed concentrates in food production and ruminant nutrition (Sanders, 2000; Watson, 2009). This increase in linoleic acid intake has resulted in increased synthesis of omega-6 (n-6) polyunsaturated fatty acid (PUFA) products and decreased synthesis of α-linolenic acid products in the body as the two fatty acids are metabolised in the same pathway (Fig. 2.1, Chapter 2) (Sprecher, 1992; Sanders, 2000; Harris, 2009). An increasing emphasis is therefore also placed on the balance between the n-6 and omega-3 (n-3) PUFA intake as well as decreasing SFA intake in the human diet (Commission of European Committees, 1992; Sprecher, 1992; EFSA, 2010).

Generally, a minimum PUFA:SFA ratio of 0.4 is recommended while 1−1.5 is considered beneficial for human health (Schmid, 2011) and an n-6:n-3 PUFA ratio of less than 4 is recommended (Simopoulos, 2004) in order to maintain a healthy balance between n-3 and n-6 metabolic products produced in the body. The meat derived from springbok generally has a favourable fatty acid profile with a PUFA:SFA ratios > 0.4 as well as n-6:n-3 PUFA < 4 and is thus considered a healthy alternative red meat (Hoffman *et al.*, 2007; Neethling *et al.*, 2018). The effect of ageing on fatty acids present in springbok meat has yet to be determined however, the reported effect of sex on individual fatty acids in springbok differs (Hoffman *et al.*, 2007; Neethling *et al.*, 2018). In general, meat derived from female springbok has higher SFA and monounsaturated fatty acid (MUFA) content than that from male springbok (mg/g of muscle) while males have higher PUFA content and PUFA:SFA ratios than females (Kroucamp, 2004; Neethling *et al.*, 2018).

In addition to their nutritional value, fatty acids present in meat can impact the flavour profile of meat. Redox reactions of fatty acids that occur during ageing give rise to volatile compounds in meat (Forss, 1969; Frankel, 2005; Ba *et al.*, 2012; Gąsior & Wojtycza, 2016). Unsaturated fatty acids have been shown to be the source of certain negative aromas and flavours in meat described as “fishy” and “rancid” (Camfield *et al.*, 1997; Wood *et al.*, 2003; Gąsior & Wojtycza, 2016; O’Sullivan, 2016). Aside from reactions involving fatty acid oxidation, volatile compounds can also arise from Maillard reactions during cooking, microbial metabolism, thiamine and ribonucleotide degradation and interactions between sugars, fats, amino acids as well as sulphur and nitrogen containing compounds (Dainty, 1985; Lawrie & Ledward, 2006; Schillinger *et al.*, 2006; Resconi *et al.*, 2013). The benefits of ageing on the flavour profile of meat are thought to stem from the production of desirable aroma and flavour compounds that occurs during this process (Lawrie & Ledward, 2006; Watanabe *et al.*, 2015).

Although no studies on the effect of ageing on both the fatty acid and volatile compound profile of aged springbok meat have been done, several studies exist for other red meat such as beef and foal (Ba *et al.*, 2014; Kim *et al.*, 2016; Sosin-Bzducha & Puchała, 2017; Maggiolino *et al.*, 2018). A significant increase in thiobarbituric acid-reactive substances (TBARS), an indicator of lipid oxidation, with ageing and storage time in beef has been reported (Sasaki *et al.*, 2001; Ba *et al.*, 2014; Sosin-Bzducha & Puchała, 2017) suggesting that lipid oxidation increases with ageing time. Additionally, individual fatty acids, particularly unsaturated fatty acids, have been reported to differ with ageing time (Sosin-Bzducha & Puchała, 2017; Holman *et al.*, 2019b). With regard to ageing method, Clausen *et al.* (2009) showed that ageing in an anaerobic system such as vacuum ageing (VAC) ageing resulted in lower TBARS than in high oxygen modified atmosphere ageing systems. These findings suggest that changes to the fatty acid profile can be expected during ageing of springbok meat.

A general increase in the number of volatile compounds produced with ageing time has been reported in beef and foal (Ba *et al.*, 2014; Maggiolino *et al.*, 2018). However, a decrease in concentration of desirable volatiles with ageing time was observed in beef (Ba *et al.*, 2014) indicating that longer ageing times could be detrimental to the eating quality of meat. Volatiles such as furan 2-pentyl, toluene and 2,4-decadienal which are primarily linked to the oxidation of lipids have been reported to differ with ageing time (Ba *et al.*, 2014; Watanabe *et al.*, 2015).

It is evident from previous studies that ageing does not indefinitely improve the flavour of meat. Furthermore, an optimal ageing time should be established to reap the benefits of the process. The highly unsaturated nature of fatty acids present in springbok meat make it particularly susceptible to the development of rancid odours as a result of lipid oxidation (Wood *et al.*, 2003; Hoffman *et al.*, 2007; Resconi *et al.*, 2013; Neethling *et al.*, 2018). Considering the impact of ageing on meat flavour as well as the health benefits linked to fatty acid composition of springbok meat, the aim of this study was to explore the effects of ageing method [skin-on and vacuum bag ageing (VAC)] and time (6, 7, 8 and 9 days) previously recommended for springbok meat (Jansen van Rensburg, 1997; North & Hoffman, 2015) on fatty acids present and volatile compounds produced in the meat. Additionally, this study aimed to establish the fatty acid and volatile compound profile of aged springbok meat.

4.3 Materials and methods

4.3.1 Harvesting, slaughter and ageing

Twenty-four springbok (twelve male and twelve female) were harvested from Brakkekuil farm in Witsand, Western Cape, South Africa according to standard operating procedures (SOP/ethical approval number SU-ACUM13-00034). The adult springbok were randomly harvested at night using a light calibre rifle fitted with a suppressor with a spotlight to immobilise the animals. A headshot was used to ensure instantaneous death. The carotid and jugular blood vessels were then severed to allow carcasses to bleed out while suspended from the transport vehicle before being transported to the onsite abattoir.

At the abattoir, all carcasses were suspended by both Achilles tendons and weighed. Thereafter, the heads and hoofs were removed and the carcasses eviscerated within two hours post mortem. The carcasses were then suspended on hooks by both Achilles tendons in a cold truck and stored overnight at 0-4°C before transportation back to the meat science laboratory at the Department of Animal Science, Stellenbosch University the next day.

At the department, the carcasses were weighed and thereafter twelve (six males and six females) springbok were randomly selected for VAC ageing and the other twelve springbok were assigned to the skin-on ageing (six males and six females) for 6, 7, 8 or 9 days (Table 3.1). The former were skinned after selection and the *Longissimus thoracis et lumborum* (LTL) muscle excised (Fig. 3.1). The muscles were immediately weighed and vacuum sealed using a Multivac vacuum sealer (Model C200, Sepp Haggenmuller, Wolfertschwenden, Germany) in a vacuum bag with the following characteristics: 70 µm polyethylene and nylon; moisture vapour transfer rate of 2.2 $g/m^2/24$ h/1 atm, O_2 permeability of 30 $cm^3/m^2/24$ h/1 atm and a CO_2 permeability of 105 $cm^3/m^2/24$ h/1 atm. The VAC samples were then left to age for the time period allocated to each animal in a chiller at 2-4°C. At the end of the designated ageing period, the VAC samples were blotted dry, weighed and the epimysium removed. The skin-on carcasses were weighed and aged in the same cold room with a relative humidity of 79-93% for the allocated ageing time period. At the end of the ageing period, the skin-on carcasses were weighed, skinned, weighed again and the LTL muscles excised. These muscles were immediately weighed, vacuum-sealed and stored along with the VAC samples in the same cold room until physical analysis the next day.

4.3.2 Sampling

The left LTL was sampled for volatile compound analysis and the right LTL for fatty acid composition. A 50 g sample portion was obtained from the *Longissimus lumborum* section of each animal's right LTL muscle, similar to that used for proximate analysis in Chapter 3 (after the ageing period). The samples were homogenised (Dampa bowl cutter, CT 35N), vacuum-sealed and stored at −80°C and analysis was carried out within 4 months after sample collection. Samples were defrosted approximately 18 h overnight at 0-4°C before analysis.

Samples for volatile compound analysis were obtained using a cylindrical corer between the 9th to 11th rib from the *Longissimus thoracis* section of the LTL muscle used for descriptive sensory analysis (prior to cooking), as described in Chapter 5. The samples were then quartered and cut into 1 cm thick portions where after 2 g samples from each muscle were individually weighed into 20 mL gas chromatography vials capped with septum caps. The samples were stored at −80°C and analysed within 30 days after sample collection. Samples were defrosted at room temperature (21°C) ca. 30 min prior to analysis.

4.3.3 Fatty acid analysis

Fat from 1 g of homogenised meat sample was extracted using 20 mL of a 2:1 (v/v) mixture of chloroform/methanol containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant (Folch *et al.*, 1957). Five mL of heptadecanoic acid (C17:0), the internal standard, was added to the mixture. The mixture was then homogenised for 30 s using a polytron mixer (WiggenHauser D-500 homogeniser, fitted with a standard shaft 1, speed-setting D). The mixture was filtered and topped up to 50 mL. A 250 µL portion of the filtrate was then dried and 2 mL of 19:1 (v/v) methanol/sulphuric acid (transmethylating reagent) added. Transmethylation was done at 70°C for 2 h followed by extraction with distilled water and hexane. The hexane layer containing the fatty acid methyl esters (FAMEs) was used for analysis.

The FAMEs were separated and analysed using a Thermo TRACE 1300 series gas-chromatograph (Thermo Electron Corporation, Milan, Italy) equipped with a flame-ionisation detector (FID). A 30 m TR-FAME capillary column with 0.25 mm internal diameter and 0.25 µm film thickness (Cat. No. HY260M142P, Anatech, Cape Town, South Africa) was used. The initial oven temperature was set at 50°C and maintained for 1 min. The temperature was then ramped up at 25°C/min to 175°C and immediately ramped up again at 1.5°C/min to 200°C and maintained here for 6 min. The final temperature increase was to 240°C at a rate of 10°C/min and maintained here for at least 2 min. The injector temperature was set at 240°C and the injection volume was set at 1 µL. The carrier gas used was hydrogen at a flow rate of 40 mL/min. FID temperature was set at 250°C.

FAMEs were identified by comparing their retention indices to those of a standard FAME mixture (Supelco™ 37 Component FAME mix, Cat no. CRM47885, Supelco, USA). Results were expressed as the concentration of the individual fatty acid (µg/g) relative to the total fatty acids present (µg/g) in a given sample. Fatty acids were also grouped according to saturation level into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and the total of the percentage fatty acids occurring in each group was calculated. PUFAs were also further grouped based on the position of their terminal double bond into omega-6 fatty acids (n-6) and omega-3 fatty acids (n-3) the sum of percentage fatty acids obtained in each group was calculated. Ratios for the PUFA to SFA and n-6 to n-3 occurring in each sample were also determined.

4.3.4 Volatile compound analysis

A 100 µL of internal standard was added to each defrosted 2 g sample. The internal standard used contained 1,2,3,4,5-pentadeuterio-6-(trideuteriomethoxy) benzene (Anisole-d8) and 3-octanol. An alkane mix containing C10 to C40 was analysed in order to calculate the retention indices as per IUPAC (1997) of the volatile compounds.

Volatile compounds were extracted by solid phase micro extraction (SMPE) using a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane fibre (Supelco 57298-U, Sigma). Extraction was done at 70°C for 10 min in a CTC auto-sampler incubator. The volatiles were then desorbed for 10 min in the 250°C GC injection port operated in pulsed splitless mode.

Separation of the compounds was by gas chromatography and identification by a coupled mass spectrometer. An Agilent 6890 N (Agilent, Palo Alto, CA) gas chromatograph coupled with an Agilent 5975B inert XL EI/CI MSD (Agilent, Palo Alto, CA) mass spectrometer was used. The carrier gas was used was Helium at a constant flow rate of 1.9 mL/min. Oven temperature was set at 70°C for 1 min increasing to 142°C at a rate of 3°C/min before being ramped up again immediately to 240°C at 5°C/min and held for 3 min. A DB-FFAP GC column (60 m, 0.25 mm internal diameter and 0.5 µm film thickness) was used. Each sample had a run time of 47.60 min.

The mass spectrometer was operated in full scan mode with a source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 280°C. The electron impact (EI) energy was set at 70 eV and data was collected in the 35-450 m/z range. Compounds were tentatively identified and confirmed using mass spectral libraries (NIST05 and Wiley spectral library collection) and retention indices (Addendum C). Compounds were presented as a ratio of the peak area of the analyte to the peak area of the internal standard (area ratio).

4.4 Statistical analysis

Statistical analysis was carried out on the means of the parameters tested using statistical software, Statistica version 13.5. Mixed model analysis of variance (ANOVA) was conducted using the VEPAC module of Statistica 13.5 and the R “lmer” package. The animals were treated as random effect while sex, ageing method and time were fixed effects in the three-way ANOVA conducted. All main and second order interaction effects were included in the model. Fisher Least Significant Difference (LSD) was used for post hoc testing. Linear correlations between the different parameters were tested in XLStat (Version 2019.1.1.56421, Addinsoft, New York, USA) using the Pearson correlation function (r).

4.5 Results

First order interactions between ageing method (M), ageing time (T) and sex (S) were observed for some fatty acids (Table 4.1); where applicable these will be discussed.

Saturated fatty acids (SFA) made up the majority of the fatty acids recorded for all treatments, followed by polyunsaturated fatty acids (PUFA) and then monounsaturated fatty acids (MUFA) (Table 4.2). Subsequently, the mean PUFA:SFA ratio across all treatments was 0.65 ± 0.42. The most abundant SFA was palmitic acid (C16:0) that made up 26.24 ± 8.35% of the fatty acids present while eicosatrienoic acid (C20:3n-3), an n-3 fatty acid, was the most abundant PUFA and the fourth most abundant fatty acid (9.94 ± 5.60%). Oleic acid (C18:1n9c) was the most abundant MUFA and the third most abundant fatty acid present (13.31 ± 9.69%). n-3 PUFA were more abundant than n-6 PUFA with a mean n-6:n-3 PUFA ratio of 0.58 ± 0.15 reported. The most abundant n-6 fatty acid was linoleic acid (C18:2n-6c) that was 8.99 ± 4.78% of the total fatty acids.

Ageing method and time (M*T) had an effect on percent dihomo-γ-linolenic acid (C20:3n-6) with VAC samples having significantly lower percent dihomo-γ-linolenic acid than skin-on samples on day 9 (Fig 4.1). Ageing method and sex (M*S) affected total n-3 PUFA content with lower total n-3 PUFA in female VAC aged samples than males (Fig. 4.2). Ageing time and sex (T*S) also interacted to yield lower percent eicosadienoic acid (C20:2n-6) in meat from 9 day aged male springbok than in meat from 8 day aged males (Fig. 4.3a). Additionally, on day 8 male springbok had higher percent eicosadienoic acid than female springbok. Arachidonic acid (C20:4n-6) was also higher in meat from male springbok on days 6 and 8 than in female springbok (Fig. 4.3b).

Ageing method alone did not have an effect on the fatty acid profile (Table 4.1) while ageing time had a significant effect on percent arachidonic acid with no arachidonic acid detected on day 9 (Table 4.2). Female springbok had higher oleic acid (C18:1n9c) and MUFA content than males while male springbok had higher linoleic acid (C18:2n-6c), arachidonic acid, eicosatrienoic acid (C20:3n-3), eicosapentaenoic acid (C20:5n-3), total PUFA, PUFA:SFA, total n-6 and n-3 PUFA content than female springbok (Table 4.2).

Table 4.1 Statistical significance of the effects of ageing method (M), time (T) and sex (S) on the percentage fatty acid profile* of springbok *Longissimus thoracis et lumborum* muscle

Fatty Acid	Method	Time	Sex	M*T	M*S	T*S
Myristic acid (C14:0)	0.489	0.942	0.170	0.362	0.385	0.690
Pentadecanoic acid (C15:0)	0.778	0.588	0.645	0.901	0.640	0.444
Palmitic acid (C16:0)	0.949	0.884	0.106	0.175	0.234	0466
Palmitoleic acid (C16:1)	0.706	0.855	0.440	0.865	0.801	0.622
Stearic acid (C18:0)	0.776	0.578	0.774	0.116	0.347	0.737
Oleic acid (C18:1n9c)	0.788	0.350	**0.022**	0.737	0.453	0.107
Linoleic acid (C18:2n-6c)	0.778	0.758	**0.011**	0.279	0.066	0.298
γ-Linolenic acid (C18:3n-6)	0.590	0.382	0.879	0.819	0.301	0.078
α-Linolenic acid (C18:3n-3)	0.749	0.910	0.240	0.539	0.522	0.503
Arachidic acid (C20:0)	0.655	0.345	0.123	0.635	0.539	0.162
Gondoic acid (C20:1)	0.675	0.631	0.502	0.465	0.152	0.220
Eicosadienoic acid (C20:2n-6)	0.527	0.433	0.184	0.516	0.116	**0.035**
Dihomo-γ-linolenic acid (C20:3n-6)	0.615	0.838	0.540	**0.021**	0.116	0.578
Arachidonic acid (C20:4n-6)	0.709	**0.001**	**0.001**	0.966	0.948	**0.019**
Eicosatrienoic acid (C20:3n-3)	0.666	0.864	**0.004**	0.137	0.033	0.100
Eicosapentaenoic acid (C20:5n-3)	0.950	0.198	**0.022**	0.380	0.222	0.176
Σ SFA	0.827	0.872	0.288	0.156	0.276	0.601
Σ MUFA	0.821	0.372	**0.026**	0.696	0.461	0.142
Σ PUFA	0.909	0.738	**0.005**	0.128	0.054	0.148
PUFA:SFA ratio	0.933	0.752	**0.007**	0.076	0.066	0.315
Σ n-6 PUFA	0.679	0.848	**0.015**	0.244	0.109	0.149
Σ n-3 PUFA	0.898	0.582	**0.003**	0.105	**0.045**	0.196
n-6:n-3 PUFA ratio	0.442	0.315	0.883	0.685	0.856	0.576

* Percentage fatty acid calculated as the percentage of an individual fatty acid in a sample (µg/g of meat) to the total fatty acids recorded in the sample (µg/g meat)

Σ SFA = %C14:0 + %C15:0 + %C16:0 + %C18:0 + %C20:0

Σ MUFA = %C16:1 + %C18:1n9c + %C20:1

Σ PUFA = %C18:2n-6c + %C18:3n-6 + %C18:3n-3 + %C20:2n-6 + %C20:3n-6 + %C20:4n-6 + %C20:3n-3 + %C20:5n-3

Σ n-6 = %C18:2n-6c + %C18:3n-6 + %C20:2n-6 + %C20:3n-6 + %C20:4n-6

Σ n-3 = %C18:3n-3 + %C20:3n-3 + %C20:5n-3

Table 4.2 Effect of ageing method, time and gender on the percentage fatty acids* of springbok *Longissimus thoracis et lumborum* muscle (mean ± standard deviation).

Fatty acids	**Method**		**Ageing time (days)**				**Sex**	
	Skin-on	VAC	6	7	8	9	Male	Female
Myristic acid (C14:0)	1.64 ± 0.82	1.78 ± 0.99	1.54 ± 0.84	1.67 ± 0.63	1.86 ± 0.86	1.75 ± 1.33	1.39 ± 0.79	2.02 ± 0.90
Pentadecanoic acid (C15:0)	0.67 ± 0.33	0.50± 0.36	0.64 ± 0.26	0.43 ± 0.22	0.83 ± 0.25	0.44 ± 0.49	0.61 ± 0.40	0.56 ± 0.30
Palmitic acid (C16:0)	27.38 ± 8.95	25.10 ± 7.93	26.02 ± 8.20	24.59 ±7.04	28.08 ± 9.42	26.28 ± 10.39	23.25 ± 8.69	29.23 ± 7.13
Palmitoleic acid (C16:1)	1.46 ± 0.88	1.81 ± 0.80	1.77 ± 1.09	1.98 ± 0.71	1.36 ± 0.70	1.43 ± 0.87	1.45 ± 0.86	1.82 ± 0.81
Stearic acid (C18:0)	24.25 ± 5.75	23.67 ± 7.18	23.23 ± 5.53	20.17 ± 4.03	25.42 ± 5.22	27.02 ± 8.98	23.89 ± 6.98	24.03 ± 5.99
Oleic acid (C18:1n9c)	13.00 ± 9.28	13.62 ± 10.48	15.21 ± 11.49	18.85 ± 8.80	10.10 ± 10.22	9.06 ± 6.51	$8.87^{b} \pm 5.21$	$17.74^{a} \pm 11.23$
Linoleic acid (C18:2n-6c)	8.68 ± 4.65	9.31 ± 5.09	9.59 ± 5.90	9.39 ± 3.54	8.33 ± 5.31	8.66 ± 5.32	$11.49^{a} \pm 4.98$	$6.49^{b} \pm 3.06$
γ-Linolenic acid (C18:3n-6)	1.20 ± 0.96	1.07 ± 1.12	0.83 ± 0.86	0.94 ± 0.68	1.25 ± 1.16	1.52 ± 1.37	1.14 ± 1.09	1.13 ± 0.99
α-Linolenic acid (C18:3n-3)	3.50 ± 2.40	3.57 ± 2.43	3.29 ± 1.89	3.73 ± 1.05	3.25 ± 2.59	3.87 ± 3.74	4.27 ± 2.79	2.80 ± 1.66
Arachidic acid (C20:0)	1.56 ± 0.73	1.59 ± 0.82	1.41 ± 0.78	1.16 ± 0.77	1.79 ± 0.74	1.94 ± 0.67	1.81 ± 0.48	1.34 ± 0.93
Gondoic acid (C20:1)	0.46 ± 0.39	0.36 ± 0.30	0.37 ± 0.34	0.42 ± 0.32	0.33 ± 0.23	0.53 ± 0.50	0.48 ± 0.41	0.35 ± 0.27
Eicosadienoic acid (C20:2n-6)	0.16 ± 0.15	0.15 ± 0.17	0.19 ± 0.11	0.20 ± 0.15	0.18 ± 0.20	0.06 ± 0.14	0.19 ± 0.18	0.12 ± 0.12
Dihomo-γ-linolenic acid (C20:3n-6)	0.62 ± 0.51	0.50 ± 0.45	0.57 ± 0.42	0.54 ± 0.39	0.66 ± 0.49	0.47 ± 0.66	0.61 ± 0.50	0.51 ± 0.46
Arachidonic acid (C20:4n-6)	0.48 ± 0.48	0.36 ± 0.40	$0.74^{x} \pm 0.41$	$0.38^{y} \pm 0.28$	$0.54^{xy} \pm 0.51$	$0.00^{z} \pm 0.00^{nd}$	$0.63^{x} \pm 0.51$	$0.21^{y} \pm 0.20$
Eicosatrienoic acid (C20:3n-3)	9.03 ± 4.22	10.85 ± 6.79	9.38 ± 5.50	10.74 ± 8.49	9.63 ± 5.03	10.03 ± 3.92	$12.94^{x} \pm 5.86$	$6.94^{y} \pm 3.42$
Eicosapentaenoic acid (C20:5n-3)	5.91 ± 2.43	5.76 ± 2.56	5.21 ± 2.50	4.81 ± 2.71	6.38 ± 2.52	6.94 ± 2.00	$6.98^{a} \pm 1.62$	$4.69^{b} \pm 2.65$
Σ SFA	55.50 ± 14.87	52.64 ± 14.69	52.85 ± 13.53	48.01 ± 9.24	57.98 ± 15.13	57.44 ± 19.89	50.95 ± 16.18	57.19 ± 12.58
Σ MUFA	14.91 ± 9.63	15.79 ± 10.98	17.36 ± 11.90	21.25 ± 9.11	11.79 ± 10.50	11.02 ± 7.15	$10.79^{b} \pm 5.70$	$19.91^{a} \pm 11.66$
Σ PUFA	29.59 ± 13.32	31.57 ± 14.98	29.80 ± 15.42	30.73 ± 14.77	30.23 ± 15.44	31.54 ± 13.87	$38.26^{x} \pm 13.74$	$22.90^{y} \pm 9.30$
PUFA:SFA	0.62 ± 0.41	0.69 ± 0.44	0.63 ± 0.44	0.70 ± 0.46	0.60 ± 0.43	0.68 ± 0.45	$0.87^{x} \pm 0.44$	$0.43^{y} \pm 0.26$
Σ n-6	11.14 ± 5.97	11.39 ± 5.70	11.91 ± 6.90	11.45 ± 4.23	10.97 ± 7.00	10.71 ± 5.88	$14.06^{a} \pm 5.98$	$8.46^{b} \pm 3.91$
Σ n-3	18.45 ± 7.66	20.18 ± 9.77	17.89 ± 8.65	19.28 ± 11.05	19.26 ± 8.68	20.83 ± 8.09	$24.19^{x} \pm 8.40$	$14.44^{y} \pm 5.78$
n-6:n-3	0.59 ± 0.17	0.57 ± 0.15	0.65 ± 0.14	0.65 ± 0.14	0.54 ± 0.18	0.49 ± 0.12	0.57 ± 0.16	0.59 ± 0.15

^{a,b} means in the same row (within the main effect) with different superscripts differ significantly from each other p ≤ 0.05

^{x,y} means in the same row (within the main effect) with different superscripts differ significantly from each other p ≤ 0.01

Table 4.2 continued

nd not detected

***** Percentage fatty acid calculated as the percentage of an individual fatty acid in a sample (µg/g of meat) to the total fatty acids recorded in the sample (µg/g meat

Σ SFA = %C14:0 + %C15:0 + %C16:0 + %C18:0 + %C20:0

Σ MUFA = %C16:1 + %C18:1n9c + %C20:1

Σ PUFA = %C18:2n-6c + %C18:3n-6 + %C18:3n-3 + %C20:2n-6 + %C20:3n-6 + %C20:4n-6 + %C20:3n-3 + %C20:5n-3

Σ n-6 = %C18:2n-6c + %C18:3n-6 + %C20:2n-6 + %C20:3n-6 + %C20:4n-6

Σ n-3 = %C18:3n-3 + %C20:3n-3 + %C20:5n-3

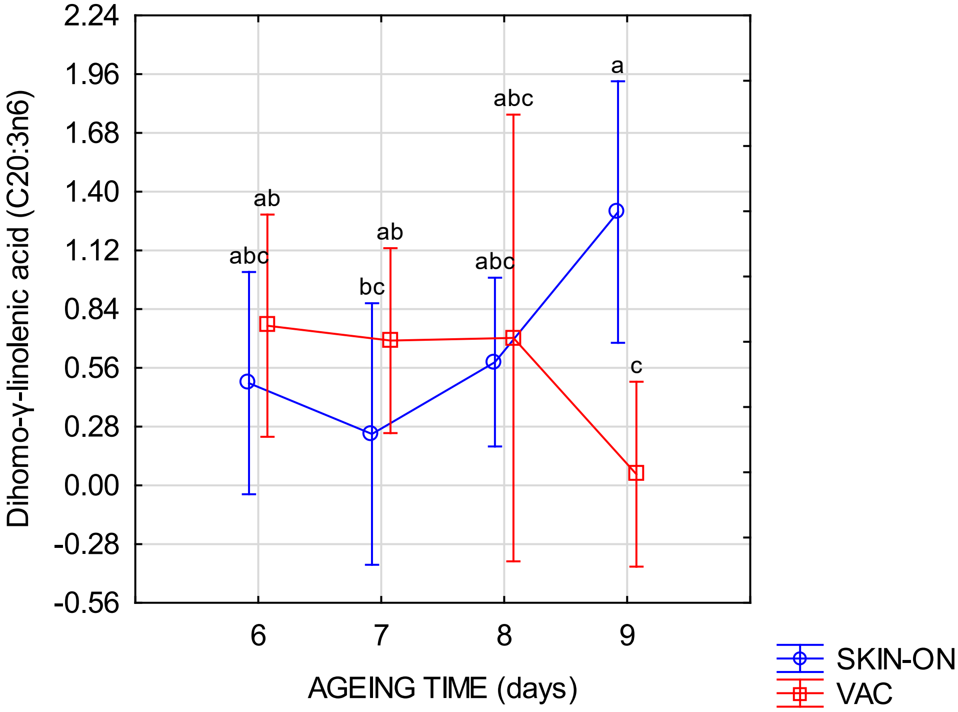

Figure 4.1 The change in mean percent Dihomo-γ-linolenic acid (C20:3n-6) in springbok *Longissimus thoracis et lumborum* muscle with ageing method and time. [a,b] Means with different superscripts differ significantly ($p \leq 0.05$). Error bars indicate 95% confidence intervals.

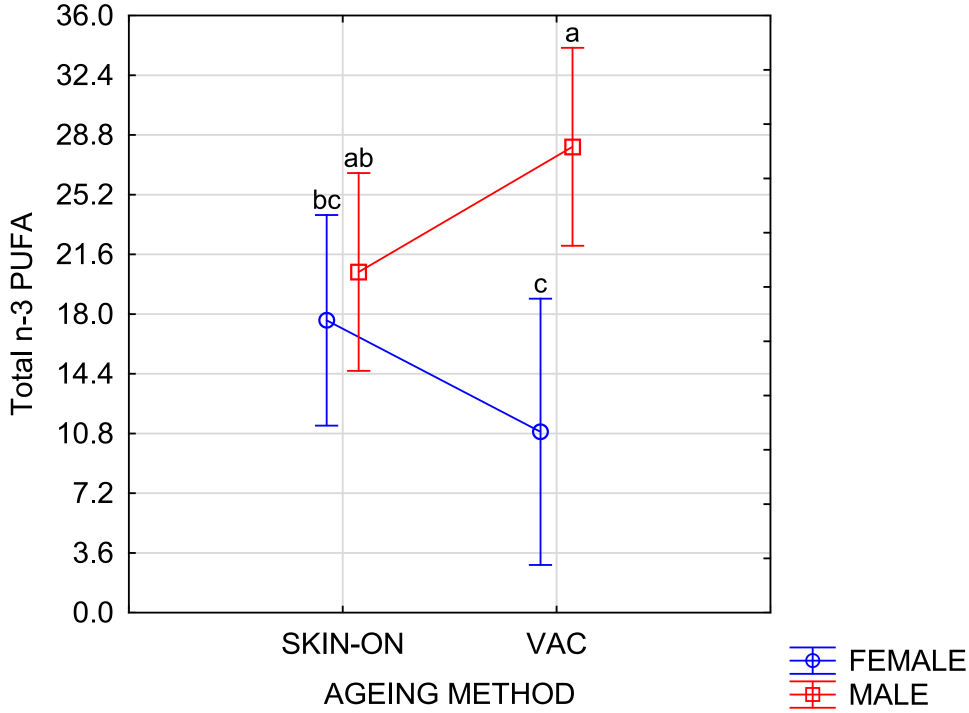

Figure 4.2 The change in mean percent total n-3 PUFA content in springbok *Longissimus thoracis et lumborum* muscle with ageing method and sex. [a,b] Means with different superscripts differ significantly (p ≤ 0.05). Error bars indicate 95% confidence intervals.

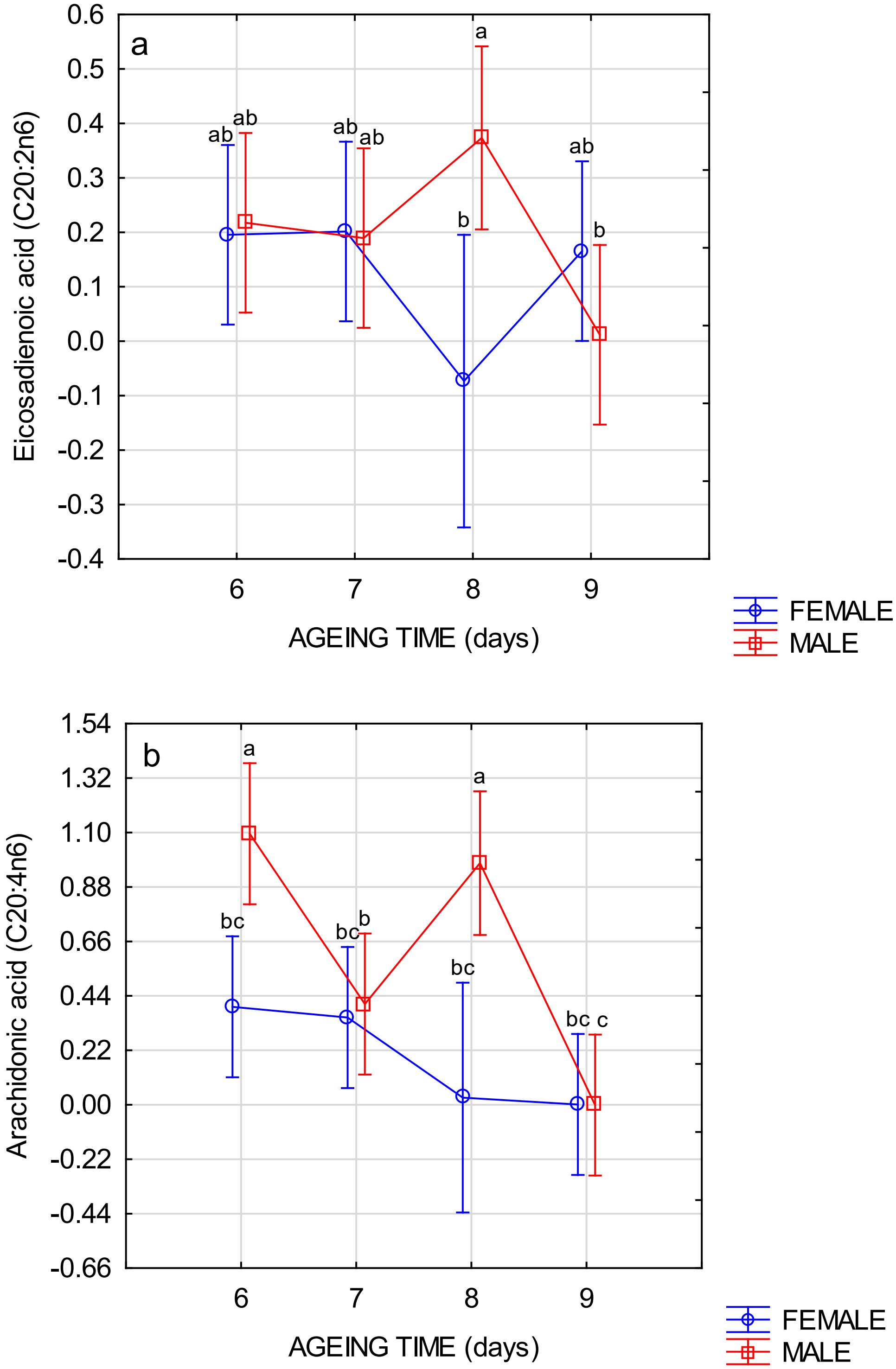

Figure 4.3 The change in mean percent eicosadienoic acid (C20:2n-6) (a) and arachidonic acid (C20:4n-6) (b) in springbok *Longissimus thoracis et lumborum* muscle with ageing time and sex. [a,b] Means with different superscripts differ significantly ($p \leq 0.05$). Error bars indicate 95% confidence intervals.

During analysis of spectra, it was observed that 3-octanol, one of the internal standards chosen for quantification, co-eluted with methyl octanoate an analyte detected in the meat samples. Consequently, peak area ratios compounds were only calculated relative to anisole d-8 which was clearly resolved in all meat samples analysed.

A total of 53 volatile compounds were identified in all the samples in this study. The majority of compounds identified were alcohols and esters (Fig. 4.4). Twelve of the detected compounds were not subjected to further statistical analysis as these compounds only occurred in a few of the samples ($n \leq 4$). The rest of the compounds reported were present in at least five samples. It is important to note that the number of samples in which a specific compound was detected (n) will vary from compound to compound as a result of specific compounds not being detected in some of the samples.

Of the volatile compounds reported, acetoin had the highest peak area ratio (Table 4.4) and was therefore the most abundant relative to the internal standard. Acetoin was higher in VAC aged samples ($p = 0.048$) as well as in female springbok samples ($p = 0.043$). Methyl hexanoate and decane also occurred in appreciable amounts (Table 4.4) although these two compounds did not differ significantly due to any of the treatments ($p > 0.05$) and have not been previously identified in fresh springbok meat (Neethling, 2016).

The interaction between ageing method and sex (M*S) had an effect on peak area ratios of furan 2-pentyl ($n = 15$), methyl nonanoate ($n = 22$), the unidentified alcohol ($n = 12$), benzyl alcohol ($n = 11$) and caprolactam ($n = 10$) ($p < 0.050$). VAC aged female springbok samples had higher peak area ratios for furan 2-pentyl (Fig. 4.5a) and an unidentified alcohol (Fig. 4.5b) than VAC aged male springbok samples and the skin-on aged samples. VAC aged male springbok samples had higher caprolactam peak area ratios than the skin-on aged samples (Fig. 4.5c). Although benzyl alcohol and methyl nonanoate were reported as significantly different, further post-hoc testing with the Games-Howell post-hoc test found no significant differences.

The interaction between ageing time and sex (T*S) affected methyl heptanoate ($n = 17$) and benzyl alcohol ($n = 11$) ($p < 0.050$) peak area ratios. On day 9, male springbok samples had higher methyl heptanoate peak area ratios than female springbok samples (Fig. 4.6). Additionally, males had higher methyl heptanoate peak area ratios on day 9 than days 6 and 7. On day 9, females had higher benzyl alcohol peak area ratios than males. However, similar to the M*S interaction observed for benzyl alcohol, further post-hoc testing revealed no significant differences.

An interaction between ageing method and time (M*T) was also reported for methyl dodecanoate ($n = 20$) and octanoic acid ($n = 14$) ($p < 0.050$) peak area ratios. Skin-on aged samples had lower methyl dodecanoate peak area ratios on day 6 than VAC aged samples while on day 7 skin on aged samples had higher methyl dodecanoate peak area ratios than VAC aged samples (Fig. 4.7a). The highest mean octanoic acid peak area ratio was recorded in day 6 skin-on aged samples (Fig. 4.7b).

Ageing time (T) affected butyrolactone ($n = 7$) and toluene ($n = 6$) peak area ratios ($p < 0.050$). Butyrolactone was not detected in day 8 and 9 samples and toluene was not detected in day 7 samples (Table 4.4). Ageing method also affected peak area ratios for toluene ($n = 6$) ($p < 0.010$), 1-octen-3-ol ($n = 24$), and acetoin ($n = 20$) peak area ratios ($p \leq 0.050$). Skin-on samples had higher peak area ratios for toluene while VAC samples had higher ratios for acetoin and 1-octen-3-ol (Table 4.4). Sex had an effect on acetoin ($n = 20$), 1-octen-3-ol ($n = 24$), 1-octanol ($n = 12$) and butyrolactone ($n = 7$) ($p < 0.050$) with female springbok having higher peak area ratios (Table 4.4).

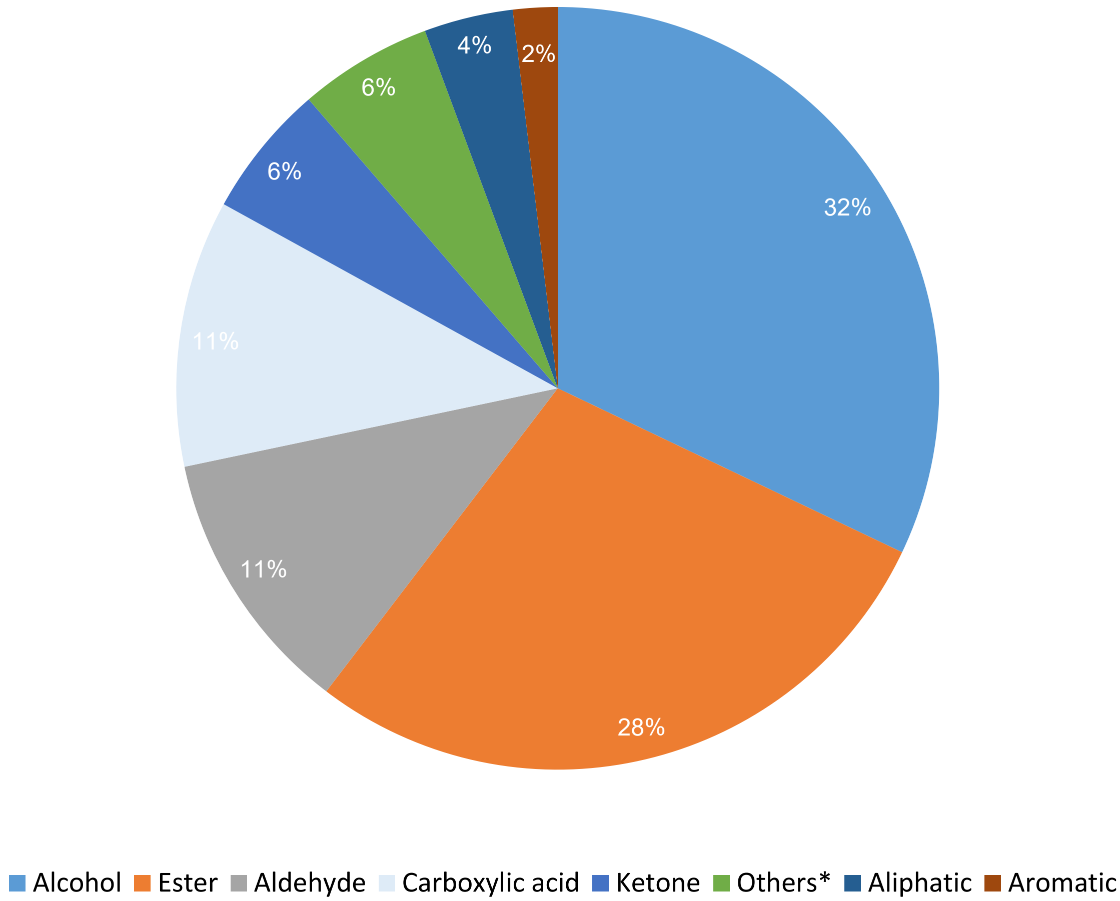

Figure 4.4 Percentage occurrence of volatile compound classes detected in aged male and female springbok *Longissimus thoracis et lumborum* muscle.

*Category contains compound with varying functional groups

Table 4.3 Retention time, aroma description from different reports and statistical significance of ageing method (M), time (T) and sex (S) and their interactions on the peak area ratios* of the volatile compound profile of a representative sample of springbok *Longissimus thoracis et lumborum* muscle

Volatile compound	Retention time (min)	Aroma description	Ageing method	Time	Sex	M*T	M*S	T*S
Decane	4.33	alkane[2]	0.478	0.446	0.569	0.811	0.359	0.187
Toluene	4.88	Strong[3,6], fruity[3], batter[3], plastic [6]	**0.008**	**0.007**	0.215	0.072	0.433	0.053
Hexanal	6.18	Apple[1], fat[1,6], fresh[1], green[1,6], oil[1]	0.290	0.714	0.331	0.801	0.342	0.610
Methyl valerate	6.33	fruit[1]	0.871	0.845	0.135	0.690	0.978	0.163
3-pentanol	7.40		0.537	0.760	0.669	0.581	0.549	0.202
2-pentanol	7.84	Fusel oil[1], green[1,2]	0.577	0.52	0.498	0.974	0.799	0.475
Methyl 4-methyl pentanoate	8.21	fruit[1]	0.703	0.318	0.292	0.759	0.541	0.815
1-butanol	8.50	fruit[1,2,6], medicine[2,6]	0.127	0.686	0.787	0.662	0.533	0.569
1-penten-3-ol	8.88	Butter[1], fish[1], green[1], oxidised[1], wet earth[1]	0.305	0.968	0.417	0.776	0.055	0.442
Methyl hexanoate	9.66	Ester[1], fresh[1], fruit[1], pineapple[1]	0.175	0.858	0.083	0.836	0.233	0.500
D-limonene	10.01	Citrus[1], mint[1]	0.484	0.415	0.082	0.711	0.888	0.553
Furan 2-pentyl	11.12	Butter[1], floral[1], fruit[1], green bean[1]	**0.027**	0.612	0.126	0.658	**0.035**	0.179
Ethyl hexanoate	11.22	Apple peel[1], brandy[1], fruit gum[1], overripe fruit[1], pineapple[1]	0.323	0.775	0.319	0.851	0.416	0.825
1-pentanol	11.74	Balsamic[1,6], fruit, green[1], pungent[1,6], yeast[1]	0.126	0.595	0.270	0.385	0.188	0.147
Methyl 2-ethyl hexanoate	12.18		0.556	0.669	0.116	0.822	0.286	0.347
Acetoin (3-hydroxybutanone)	12.56	Buttery[1], creamy[1], green pepper[1]	**0.048**	0.728	**0.043**	0.892	0.147	0.119
Methyl heptanoate	12.95	green[1]	0.177	0.886	0.510	0.390	0.226	**0.034**
Methyl 2-hydroxypropanoate	13.61		0.340	0.366	0.117	0.825	0.281	0.119
1-hexanol	14.76	Banana[1], flower[1], grass[1], herb[1]	0.095	0.379	0.074	0.826	0.174	0.408
Methyl octanoate**	15.97	Fruity[1], orange[1], wax[1], wine[1]	0.703	0.610	0.592	0.761	0.952	0.390
1-octen-3-ol	17.51	Cucumber[1], earth[1,6], fat[1], floral[1], mushroom[1,4,6], metallic[4]	**0.050**	0.495	**0.037**	0.386	0.063	0.678
1-heptanol	17.60	herb[2]	0.733	0.633	0.997	0.369	0.530	0.870
1-hexanol-2-ethyl	18.55	Green[1], rose[1]	0.838	0.842	0.428	0.483	0.546	0.267
Methyl nonanoate	18.79	Coconut[1], floral[1], fruit[1]	0.099	0.141	0.205	0.595	**0.013**	0.075
1-octanol	20.24	Bitter almond[1], burnt matched[1], fat[1], floral[1]	0.607	0.631	**0.033**	0.207	0.667	0.924

Table 4.3 continued

Volatile compound	Retention time (min)	Aroma description	Ageing method	Time	Sex	M*T	M*S	T*S
3,5-octadien-2-one	20.41	green[1]	0.076	0.547	0.118	0.664	0.143	0.611
2,3-butanediol	20.42	Sulphur[5], onion[2,5]	0.678	0.886	0.817	0.783	0.914	0.263
Methyl decanoate	21.39	wine[2]	0.714	0.508	0.107	0.323	0.211	0.367
Butyrolactone	21.49	Caramel[1], cheese[1], roasted nut[1]	0.353	**0.035**	**0.027**	0.941	0.148	0.066
2-octen-1-ol	21.57	floral[1]	0.118	0.681	0.058	0.403	0.131	0.908
Unidentified alcohol	22.19	-	**0.034**	0.829	**0.021**	0.909	**0.024**	0.427
2,4- decadienal	26.02	seaweed[2], fatty[6], rancid[6]	0.532	0.927	0.269	0.551	0.613	0.669
Methyl dodecanoate	26.18	-	0.791	0.524	0.096	**0.021**	0.145	0.312
Hexanoic acid	26.75	Cheese[1], oil[1], pungent[1], sour[1]	0.453	0.766	0.519	0.808	0.516	0.646
Benzyl alcohol	27.15	Boiled cherries[1], moss[1], roasted bread[1], rose[1]	**0.021**	**0.046**	0.186	0.078	**0.015**	**0.040**
Dimethyl sulphone	27.48	Sulphur[2], burnt[2]	0.941	0.500	0.336	0.886	0.751	0.393
Hexanoic acid, 2-ethyl	28.94	Green[1], rose[1]	0.760	0.222	0.059	0.841	0.596	0.070
Methyl tetradecanoate	30.52	Fat[1], orris[1]	0.981	0.827	0.637	0.857	0.713	0.690
Octanoic acid	31.27	Cheese[1], fat[1], grass[1], oil[1]	**0.047**	**0.013**	0.257	**0.041**	0.148	0.228
Caprolactam	32.83	spice[1]	0.747	0.215	0.373	0.069	**0.033**	0.205
Methyl hexadecanoate	34.49	-	0.602	0.877	0.438	0.835	0.445	0.275

*Peak area ratios calculated as a ratio of the analyte to the internal standard, anisole-d8 present at 1ppm during analysis

** Co-elution observed between methyl octanoate and 3-octanol (potential internal standard)

[1] https://www.femaflavor.org/; [2] http://www.flavornet.org/flavornet.html; [3](MacLeod & Coppock, 1976); [4] (Peterson & Chang, 1982);[5](Jordán *et al.*, 2003);[6](García-González *et al.*, 2008).

Table 4.4 Effect of ageing method, time and gender on the peak area ratios* of volatile compounds detected in a representative sample of springbok *Longissimus thoracis et lumborum* muscle (means ± standard deviation).

Volatile compound	**Ageing method**		**Ageing time (days)**				**Sex**	
	Skin-on	VAC	6	7	8	9	Male	Female
Decane	0.15±0.06	0.14±0.05	0.14±0.02	0.13±0.02	0.18±0.06	0.14±0.09	0.14±0.03	0.15±0.07
Toluene	0.02^{x} ±0.02	0.01^{y}±0.01	0.01^{xy} ±0.02	0.00^{y} ±0.00nd	0.01^{y} ±0.01	0.02^{x} ±0.03	0.01±0.02	0.01±0.02
Hexanal	0.01±0.00	0.01±0.01	0.01±0.02	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01
Methyl valerate	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.00	0.01±0.00	0.01±0.01
3-pentanol	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01
2-pentanol	0.02±0.02	0.02±0.02	0.01±0.01	0.02±0.02	0.02±0.03	0.01±0.02	0.02±0.01	0.02±0.02
Methyl 4-methyl pentanoate	0.00±0.00	0.00±0.00	0.00±0.00nd	0.00±0.00	0.00±0.00	0.00±0.00nd	0.00±0.00	0.00±0.00
1-butanol	0.01±0.01	0.02±0.01	0.02±0.02	0.02±0.02	0.02±0.03	0.02±0.01	0.02±0.01	0.02±0.02
1-penten-3-ol	0.00±0.01	0.01±0.02	0.01±0.02	0.01±0.02	0.00±0.00nd	0.01±0.01	0.01±0.01	0.01±0.02
Methyl hexanoate	0.47±0.37	0.75±0.74	0.60±0.92	0.63±0.70	0.65±0.43	0.57±0.32	0.36±0.24	0.87±0.73
D-limonene	0.02±0.02	0.02±0.02	0.01±0.01	0.02±0.02	0.02±0.01	0.01±0.02	0.01±0.01	0.02±0.02
Furan 2-pentyl	0.03^{b} ±0.03	0.08^{a} ±0.09	0.07±0.10	0.06±0.09	0.04±0.04	0.05±0.04	0.04±0.03	0.07±0.09
Ethyl hexanoate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1-pentanol	0.02±0.01	0.02±0.01	0.02± 0.01	0.02±0.05	0.02±0.01	0.02±0.01	0.01±0.01	0.02±0.01
Methyl 2-ethyl hexanoate	0.01±0.01	0.01±0.01	0.01±0.00	0.01±0.01	0.01±0.01	0.00±0.00	0.00±0.00	0.01±0.01
Acetoin	0.50^{b} ±0.40	0.87^{a} ±0.67	0.01±0.67	0.02±0.75	0.01±0.34	0.01±0.58	0.43^{b} ±0.28	0.90^{a} ±0.71
Methyl heptanoate	0.01±0.01	0.00±0.01	0.01±0.01	0.00±0.00	0.01±0.00	0.01±0.01	0.01±0.01	0.00±0.01
Methyl 2-hydroxypropanoate	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01
1-hexanol	0.01±0.02	0.03±0.05	0.03±0.06	0.02±0.04	0.03±0.02	0.00±0.01	0.01±0.01	0.04±0.05
Methyl octanoate**	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.01	0.01±0.00	0.00±0.00
1-octen-3-ol	0.04^{b} ±0.02	0.07^{a} ±0.05	0.06±0.07	0.04±0.05	0.05±0.02	0.06±0.02	0.03^{b} ±0.02	0.07^{a} ±0.05
1-heptanol	0.00±0.01	0.00±0.01	0.00±0.00nd	0.00±0.01	0.00±0.01	0.01±0.01	0.00±0.01	0.00±0.01
1-hexanol-2-ethyl	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Methyl nonanoate	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.0	0.01±0.01	0.01±0.01	0.01±0.00	0.02±0.01
1-octanol	0.01±0.02	0.01±0.02	0.01±0.02	0.01±0.02	0.01±0.01	0.02±0.02	0.00±0.01^{b}	0.02^{a} ±0.02
3,5-octadien-2-one	0.00±0.00nd	0.00±0.01	0.01±0.01	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.01
2,3-butanediol	0.00±0.01	0.01±0.02	0.01±0.01	0.01±0.01	0.00±0.01	0.01±0.01	0.01±0.01	0.01±0.01
Methyl decanoate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 4.4 continued

Volatile compounds	Ageing method		Ageing time (days)				Sex	
	Skin-on	VAC	6	7	8	9	Male	Female
Butyrolactone	0.00±0.01	0.01±0.01	0.01[a] ±0.01	0.01[ab] ±0.01	0.00[b] ±0.00[nd]	0.00[b] ±0.00[nd]	0.00[b] ±0.00	0.01[a] ±0.01
2-octen-1-ol	0.00±0.01	0.01±0.01	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.01	0.02±0.01	0.01±0.01
Unidentified alcohol	0.00[b] ±0.00	0.00[a] ±0.00[a]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00[b] ±0.00	0.00[a] ±0.00
2,4- decadienal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Methyl dodecanoate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Hexanoic acid	0.01±0.02	0.01±0.18	0.11±0.25	0.02±0.02	0.01±0.02	0.03±0.03	0.02±0.02	0.07±0.18
Benzyl alcohol	0.01[a] ±0.00	0.00[b] ±0.00	0.01±0.01	0.00±0.01	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00
Dimethyl sulphone	0.01±0.02	0.02±0.04	0.03±0.06	0.01±0.02	0.01±0.01	0.00±0.01	0.00±0.01	0.02±0.04
Hexanoic acid, 2-ethyl	0.00±0.00	0.00±0.00	0.00±0.01	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Methyl tetradecanoate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Octanoic acid	0.00[a] ±0.01	0.00[b] ±0.00	0.01[a] ±0.01	0.00[b] ±0.00	0.00[b] ±0.00	0.00[b] ±0.00	0.00±0.00	0.00±0.01
Caprolactam	0.01±0.02	0.02±0.03	0.01±0.02	0.02±0.03	0.01±0.01	0.03±0.04	0.02±0.03	0.01±0.02
Methyl hexadecanoate	0.02±0.01	0.02±0.02	0.01±0.01	0.02±0.03	0.02±0.01	0.02±0.01	0.02±0.02	0.02±0.01

[a,b] means in the same row (within the main effect) with different superscripts differ significantly from each other $p \leq 0.05$

[x,y] means in the same row (within the main effect) with different superscripts differ significantly from each other $p \leq 0.01$

*Peak area ratios calculated as a ratio of the analyte to the internal standard, anisole-d8 present at 1ppm during analysis

** Coelution observed between methyl octanoate and 3-octanol (potential internal standard)

[nd] analyte not detected in samples

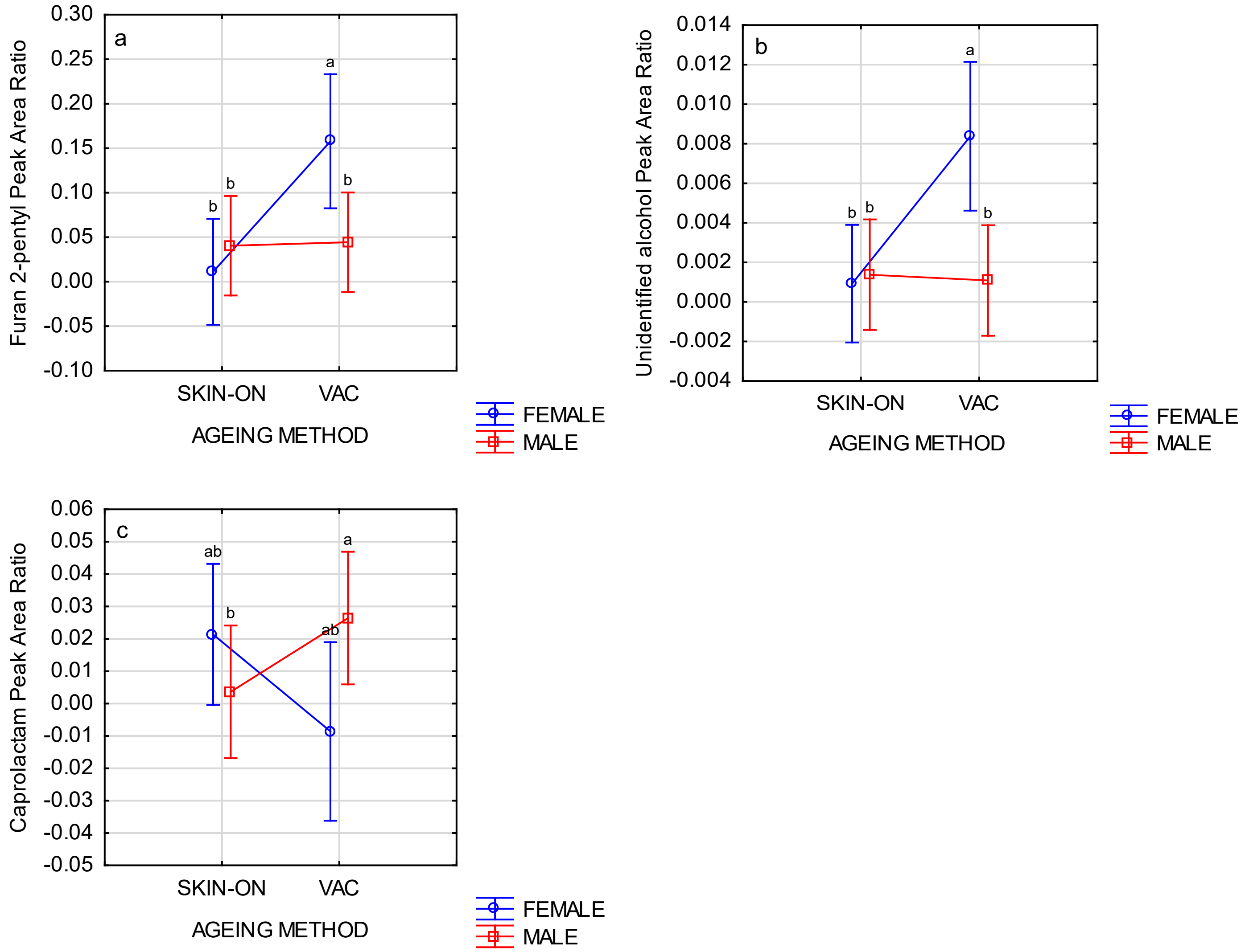

Figure 4.5 The mean peak area ratios for furan 2-pentyl (a), the unidentified alcohol (b) and caprolactam (c) detected in springbok *Longissimus thoracis et lumborum* muscle affected by ageing method and sex. [a,b] Means with different superscripts differ significantly ($p \leq 0.050$). Error bars indicate 95% confidence intervals.

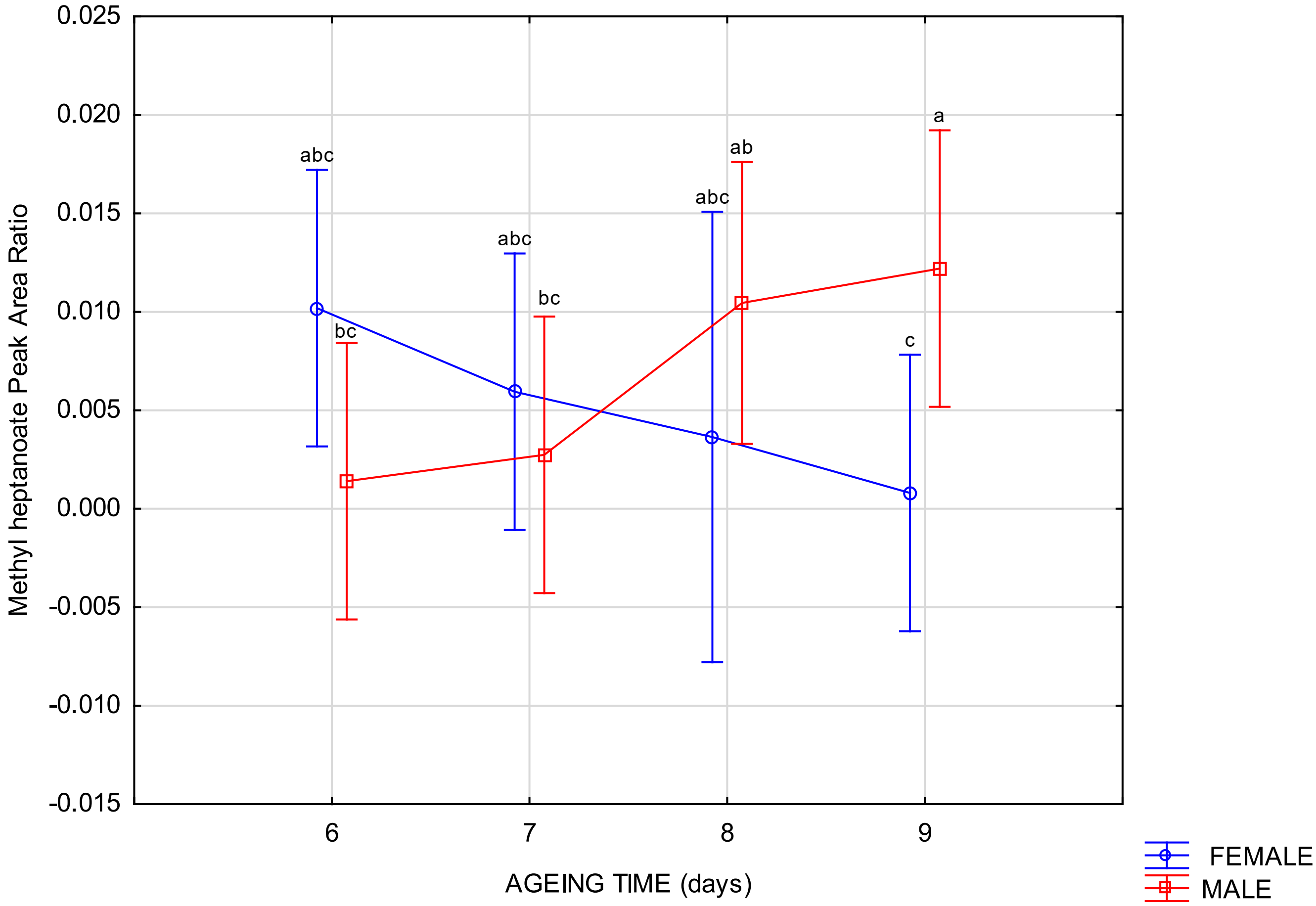

Figure 4.6 The interaction between ageing method and sex for mean methyl heptanoate peak area ratio in springbok *Longissimus thoracis et lumborum* muscle. [a,b] Means with different superscripts differ significantly ($p \leq 0.050$). Error bars indicate 95% confidence intervals.

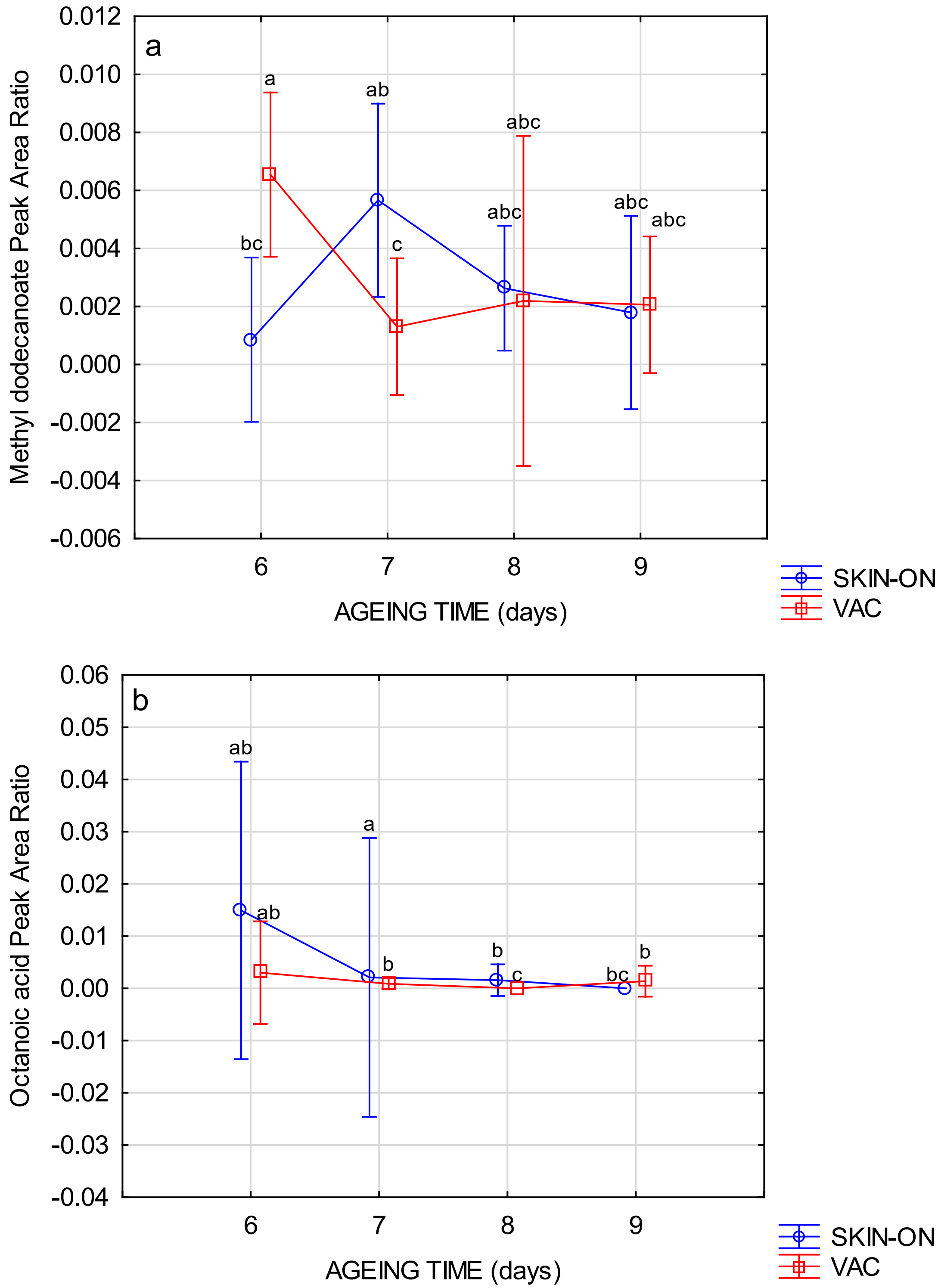

Figure 4.7 The interaction between ageing method and time for mean methyl decanoate (a) and octanoic acid (b) peak area ratio in springbok *Longissimus thoracis et lumborum* muscle. [a,b] Means with different superscripts differ significantly (p ≤ 0.050). Error bars indicate 95% confidence intervals.

4.6 Discussion

There were some differences noted between the fatty acid profile of aged springbok in the current study and findings from fresh springbok in previous studies. The current study found a lower contribution of unsaturated fatty acids to the overall fatty acid profile (Table 4.2) than previously found in fresh springbok meat (Hoffman *et al.*, 2007; Neethling, 2016) suggesting that ageing resulted in a decline of unsaturated fatty acids. This was expected as unsaturated fatty acids are more susceptible to oxidation during the ageing period due to the double bonds present (Wood & Enser, 1997; Shen *et al.*, 2012; Matarneh *et al.*, 2017). However, samples of fresh (day 1) LTL samples could not be collected during the current study to verify this.

The total fatty acid content of aged springbok in the current study (23.68 ± 7.84 mg/g of muscle) was higher than that in fresh springbok meat (8.052 to 11.841 mg/g of muscle) (Kroucamp, 2004). This is likely because of a higher fat content in the current study (2.37 ± 0.78%) than in the latter (2.20%) and not necessarily the ageing process. Previous studies have reported higher fatty acid content in beef breeds with higher IMF (Frank *et al.*, 2016) as well as a significant correlation between total fatty acids (mg/g of muscle) and IMF ($r = 0.66$; $p < 0.01$) in fresh springbok LTL muscle (Hoffman *et al.*, 2007). It therefore stands to reason that since females had higher IMF content than males in the current study ($p = 0.004$), the total fatty acid content would also differ. This was indeed the case with female springbok (28.29 ± 8.21 mg/g of muscle) having higher fatty acid content ($p = 0.004$) than male springbok (19.08 ± 3.83 mg/g of muscle) (Addendum B).

When further examining the effect of IMF content on fatty acid content (mg/g of muscle), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1n9c) were higher in aged springbok in the current study as compared to fresh springbok in the study by Hoffman *et al.* (2007). However, the same fatty acids were lower in aged springbok than fresh springbok from the study by Neethling *et al.* (2018) where the mean IMF content was 3.07 ± 0.02%. Positive correlations between fatty acid content and IMF content in fresh springbok were established for palmitic acid (C16:0) ($r = 0.73$; $p < 0.01$), stearic acid (C18:0) ($r = 0.69$; $p < 0.01$) and oleic acid (C18:1n9c) ($r = 0.72$; $p < 0.01$), suggesting that the differences in these fatty acids between aged and fresh springbok were primarily driven by differences in IMF content and not the ageing process itself. Therefore, to better understand the changes that occur to individual fatty acids in aged springbok compared to fresh springbok, one would have to compare the fatty acid profile of fresh and aged muscles from the same animals.

The mean PUFA:SFA ratio (0.65 ± 0.42) was above the recommended minimum ratio of 0.4 (Wood *et al.*, 2003) but below the 1-1.5, ratio considered to be beneficial for human health (Schmid, 2011). Sex impacted the PUFA:SFA ratio ($p = 0.007$) with male springbok having a higher mean PUFA:SFA than the female springbok (Table 4.2). Additionally, half the female springbok fell below the recommended minimum PUFA:SFA ratio of 0.4 (Table 4.2) while half the male springbok exhibited PUFA:SFA ratios > 1 (considered beneficial for human health) indicating that in these samples, PUFA dominated the fatty acid profile. Significant correlations were also found between PUFA:SFA ratio and myristic acid (C14:0) ($r = -0.761$, $p < 0.0001$), palmitic acid (C16:0) ($r = -0.872$, $p < 0.0001$), stearic acid (C18:0) ($r = -0.629$, $p = 0.001$), linoleic acid (C18:2n-6c) ($r = 0.952$, < 0.0001), α-linolenic acid (C18:3n-3) ($r = 0.695$, $p = 0.0002$) arachidonic acid (C20:4n-6) ($r = 0.663$, $p = 0.0004$) and eicosatrienoic acid (C20:3n-3) ($r = 0.913$, $p < 0.0001$) suggesting that these fatty acids contributed most to the PUFA:SFA ratio. Of the fatty acids listed that correlated with the PUFA:SFA ratio, only linoleic acid (C18:2n-6c) and eicosatrienoic acid (C20:3n-3) differed significantly between males and females (Table 4.1) suggesting that these fatty acids greatly influenced the differences in PUFA:SFA ratio reported between male and female springbok.

The differences in PUFA:SFA as well as in individual and total PUFA content between the sexes can again be traced back to the difference in IMF content between males and females, as discussed earlier. A chloroform/methanol extraction of fat extracts both triacylglycerides (TAGs) from adipose tissue as well as phospholipids from cell membranes (Wood, 2017). Lower IMF content has been linked to a higher phospholipid contribution to the IMF content (Clausen *et al.*, 2009) therefore male springbok can be said to have a higher phospholipid contribution to the IMF content than female springbok. Phospholipids portion of extracted fat have been shown to possess a greater proportion of PUFAs than the TAG portions (Wood *et al.*, 2008; Resconi *et al.*, 2013; López-Bote, 2017; Wood, 2017); thus the reason male springbok (mean IMF = 1.9 ± 0.38%) had higher PUFA:SFA ratios and total PUFA content than female springbok (mean IMF = 2.8 ± 0.82%).

The mean n-6:n-3 PUFA ratio was 0.58 ± 0.15 indicating that n-3 fatty acids are more abundant than n-6 fatty acids in springbok meat. The mean n-6:n-3 PUFA ratio was also well below the minimum of 4, the recommended ratio for a healthy diet (Simopoulos, 2004). Ageing springbok meat regardless of method and time implemented in the current study therefore did not negatively impact the beneficial n-6:n-3 PUFA ratio of springbok meat (Table 4.2).

Although the total percentages of n-6 and n-3 fatty acids were higher in male springbok than in female springbok (Table 4.2) ($p = 0.015$ and $p = 0.003$, respectively), the n-6:n-3 ratio did not differ significantly between the two sexes ($p = 0.883$). Additionally, the n-6 and n-3 content did not differ ($p = 0.449$ and $p = 0.167$, respectively) between male and female springbok when considering the mg/g of muscle content (Addendum B). Since males had higher PUFA content and therefore greater percentage contribution of PUFAs to total fatty acid content, it stands to reason that males would have significantly higher percentage totals for n-6 and n-3 PUFAs. The absolute amount of n-6 and n-3 (mg/g of muscle) therefore did not differ between the sexes (Addendum B). However, their contribution to the overall fatty acid profile (percent composition) differed (Table 4.2).

Ageing time has been reported to affect the concentration of individual fatty acids (Sosin-Bzducha & Puchała, 2017; Holman *et al.*, 2019a) as a result of oxidative processes (Watanabe *et al.*, 2015; Sosin-Bzducha & Puchała, 2017). Of the fatty acids identified in the current trial, three differed with ageing time; dihomo-γ-linolenic acid (C20:3n-6) (M*T), eicosadienoic acid (C20:2n-6) and arachidonic acid (T*S) (C20:4n-6) ($p < 0.05$); all n-6 PUFA and had some significant differences reported on day 9 (Figs. 4.1 & 4.3, respectively). These n-6 PUFAs are readily synthesized in the human body from linoleic acid (C18:2n-6c) due to the abundance of the latter in the human diet (Sanders, 2000; Dupont, 2006). Reduced intake of n-6 PUFAs could potentially be favourable in increasing the synthesis of long chain n-3 PUFA from α-linolenic acid (C18:3n-3) and their subsequent products (Fig. 2.1, Chapter 2) such as 5-series leukotrienes, 3-series prostaglandins and thromboxanes (Harris, 2009).

The 53 volatile compounds detected in the current study exceeded the 26 previously detected in fresh springbok meat (Neethling, 2016). Carboxylic acids of longer chain length (C4 to C9) were detected in aged springbok meat as compared to fresh springbok meat (C2 to C7) (Neethling, 2016). Nonetheless, similar alcohols were detected in both aged and fresh springbok meat (1-pentanol, 1-hexanol, 1-heptanol, 1-octen-3-ol, 1-octanol and 2,3-butanediol) in addition to several other alcohols only detected in aged springbok meat (benzyl alcohol, 1,4-butanediol, 2-penten-1-ol, 3-methyl-2-butanol, 3-pentanol, 2-pentanol, 1-butanol). As saturated and unsaturated alcohols and carbonyls arise from the oxidation of unsaturated fatty acids (Whitfield, 1992; Resconi *et al.*, 2013), lipid oxidation during the ageing process could be considered the primary reason for the formation of more alcohols and carbonyl compounds in aged as compared to fresh springbok meat. Similar ketones were present in fresh and aged springbok meat although 3,5-octadien-2-one was only present in aged springbok meat. While only two esters were identified in fresh springbok meat (Neethling, 2016), fifteen were identified in aged springbok meat, the majority of which were methyl esters. Dimethyl sulphone and furan 2-pentyl were present in both fresh and aged meat samples. It is evident that in springbok as with meat from other species, the ageing process facilitates increased volatile compound production (Watanabe *et al.*, 2015; Maggiolino *et al.*, 2018).

Due to sheer frequency of occurrence (Fig 4.4), one would expect that alcohols and esters would contribute greatly to the flavour profile in the current study. However, in general alcohols as well as fatty (carboxylic) acids have been reported to have high detection and recognition thresholds in water (> 100 µg/L) while esters had relatively lower detection and recognition thresholds (Czerny *et al.*, 2008) suggesting esters would significantly contribute to the aroma profile. Aldehydes have also been noted to have low odour thresholds thereby playing a major role in flavour perception (Brewer & Vega, 1995; Wood *et al.*, 2003). As information on the impact of these compounds on flavour in springbok meat is lacking (i.e. absolute quantities, aroma description in springbok meat, interactions between compounds and detection and recognition thresholds), further investigation of the impact of the identified volatiles on the flavour profile is recommended.

The higher acetoin content observed in meat from female springbok (Table 4.4) is similar to findings in fresh springbok (Neethling, 2016). Although positive correlations between acetoin and gamey aroma and flavour ($r = 0.781$; $p = 0.003$ and $r = 0.742$; $p = 0.006$, respectively) and herbaceous aroma and flavour ($r = 0.604$; $p = 0.038$ and $r = 0.613$; $p = 0.034$, respectively) in meat from several game species, including springbok have been reported, these correlations were postulated to be coincidental (Neethling, 2016). Additionally, such strong significant correlations with gamey aroma or flavour descriptive sensory analysis scores (DSA) were not found in the current study ($r = -0.026$; $p = 0.903$ and $r = 0.211$; $p = 0.322$, respectively). Acetoin has also been shown to play a significant role in beef flavour with the compound being identified as most closely linked to overall flavour desirability scores ($r = 0.57$, $p < 0.01$) as found in a consumer panel (O'Quinn *et al.*, 2016). Acetoin was further linked to positive attributes in beef such as grilled flavour ($r = 0.54$, $p < 0.01$) and negatively correlated to negative attributes in beef such as gamey flavour ($r = -0.47$, $p < 0.01$) and livery flavour ($r = -0.54$, $p < 0.01$) (O'Quinn *et al.*, 2016). However, other researchers have described aromas related to acetoin in meat as "non-fresh" and being associated with "cheesy" odour in spoiling meat (Dainty, 1985; Casaburi *et al.*, 2015). Although acetoin's role in flavour perception in beef has been shown to be noticeable (O'Quinn *et al.*, 2016), the lack of correlation with the major flavour attributes of game meat suggest that acetoin does not contribute greatly to springbok meat flavour despite being the most abundant volatile compound detected. Gas chromatographic analysis coupled with olfactometric detection (GC-O) could be applied to better understand the role of acetoin in springbok meat flavour.

The formation of furan 2-pentyl has been attributed to the autoxidation of n-6 PUFAs such as linoleic acid (Krishnamurthy *et al.*, 1967; Elmore *et al.*, 1999; Resconi *et al.*, 2013). The higher occurrence of furan 2-pentyl in female VAC samples (Fig. 4.4a) can be explained by the corresponding lower values of linoleic acid (C18:2n-6c) ($p = 0.066$), as well as eicosadienoic acid (C20:2n-6) ($p = 0.116$), and total n-6 PUFA ($p = 0.109$) in these samples. A significant negative correlation was however only identified between furan 2-pentyl and total n-6 PUFA ($r = -0.416$; $p = 0.043$). Furans are reported to have relatively high odour threshold values and thus do not contribute as greatly to the characteristic flavour in edible oils and cooked meat (Evans *et al.*, 1971; Elmore *et al.*, 1999). It is therefore unlikely that a higher occurrence of furan 2-pentyl in VAC female meat samples would translate significantly in the subsequent sensory analysis. Nonetheless furan 2-pentyl can be considered for further studies as an indicator of n-6 PUFA decline in springbok meat.

Octanoic acid, which was found to be present in fourteen samples during volatile compound analysis was only present in five samples during fatty acid analysis. During volatile compound identification, the initial dominant fragments identified for octanoic acid was either 101 or 115 which have both been previously reported for octanoic acid (National Institute of Standards and Technology, 2018). This highlights some of the shortcomings related to GC analysis especially when it comes to profiling work. Differences in compound extraction methods, detector used and time of analysis could be the cause of the differences in octanoic acid detection between studies. For the volatile compound analysis, SPME was used for extraction coupled with a mass spectrometer was used for compound detection and analysis was done within 30 days after ageing was completed. In the case of fatty acid analysis, fatty acids were esterified to form methyl esters for quantification using an FID detector and was done more than three months after ageing was completed. Making definitive conclusions on the effect of ageing on octanoic acid from this study would therefore be questionable. However, it is important to note that in both analyses (fatty acid and volatile compound), octanoic acid detected in the samples was in relatively low quantities. External standards and retention indices can be used to aid confirmation of volatile compounds detected.

Volatile compound profiles can give an indication of the various processes occurring in meat that affect its flavour. Further studies on the volatile profile of both aged and fresh springbok meat should be aimed at confirming the volatile compounds identified in this study as well as previous studies (Neethling, 2016). From the compounds identified in the current study, external standards can be prepared for the accurate identification and absolute quantification of these compounds in future studies. Additionally, use of internal standards that more accurately represent the different classes of compounds expected can be used. Finally, for a clearer link between volatile compounds and the sensory attributes observed (Chapter 5), application of gas chromatographic analysis coupled with olfactometric detection (GC-O) should be considered (Brattoli *et al.*, 2013).

4.7 Conclusion

The ageing methods and times recommended for springbok meat did not negatively impact PUFA:SFA and n-6:n-3 PUFA ratios as mean values for these two were still within the recommended range for a healthy diet. A decline in n-6 PUFAs with 9 day ageing was noted and could potentially be linked to health benefits linked with lower n-6 PUFA intake and better n-3 PUFA uptake. The inherent differences in IMF content of males and females translates to differences in several unsaturated fatty acids during ageing. Intermuscular fat content seemed to be the main cause of differences noted between findings in the current study and previous studies on the fatty acid profile of fresh springbok therefore to better understand how ageing time affects fatty acid profile, muscles from the same animals should be compared to each other.

The volatile compounds tentatively identified in this study show that ageing results in the formation of more volatile compounds than in fresh springbok meat. Additionally, there was a higher occurrence of compounds typically linked to lipid oxidation. Due to the semi-quantitative method employed in the study, definitive conclusions of the effect of ageing method and time on specific volatiles cannot be drawn. However, results from this study can aid when selecting external and internal standards during future analyses of the volatile profile of springbok meat.

4.8 References

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CHAPTER 5

The effects of ageing method and time on the sensory profile of springbok (*Antidorcas marsupialis*) *Longissimus thoracis et lumborum* muscle

5.1 Abstract

This study determined the effect of ageing method [skin-on and vacuum bag ageing (VAC)] and ageing time (6, 7, 8 and 9 days) on the sensory profile of the *Longissimus thoracis et lumborum* (LTL) muscles of 24 springbok (12 males and 12 females). Several first order interactions between the treatments applied were observed. Overall aroma intensity, gamey aroma, tenderness and mealiness scores on day 6 were higher ($p < 0.05$) in skin-on aged samples than VAC aged samples. Day 7 skin on aged samples had the lowest scores ($p < 0.05$) for initial juiciness, tenderness and mealiness as well as the highest ($p < 0.05$) residue scores and Warner Bratzler Shear Force (WBSF) values. Interactions between ageing method and sex (M*S) significantly impacted mean scores of aroma attributes beef-like aroma, metallic aroma, sweet-associated aroma and sour-associated aroma while interactions between ageing time and sex (T*S) were noted for gamey aroma, beef-like aroma, sweet-associated aroma, liver-like flavour, tenderness, mealiness and residue scores ($p < 0.05$). Significant correlations ($p < 0.05$) established between metallic, sweet-associated and sour-associated aromas suggests that perception of these attributes is interlinked. The positive correlations established between gamey aroma and liver-like aroma ($r = 0.620$; $p = 0.002$), metallic aroma ($r = 0.687$; $p = 0.0003$) and sour-associated aroma ($r = 0.807$; $p < 0.0001$) suggest that these attributes characterise the aroma profile of aged springbok. There were few differences in flavour attribute scores suggesting that the recommended ageing methods were capable of producing meat with consistent flavour attributes. In general, skin-on ageing seemed to provide clearer consistent trends for attributes than VAC ageing did. Lower scores for gamey associated attributes in 6 days VAC aged meat suggest the method can be used to produce springbok meat with milder gamey attributes.

Keywords: Springbok, skin-on ageing, game meat

5.2 Introduction

When one considers the myriad of changes that can take place during the ageing of meat, it is almost surprising that consistent meat quality can still be achieved. Lipolysis, lipid oxidation, proteolysis and microbial metabolic activities are some of the processes during ageing that can impact the flavour of meat (Lawrie & Ledward, 2006; Flores, 2017). The resulting eating quality of meat should therefore not be overlooked when optimising the ageing process. This is particularly important with game species as South African consumers have highlighted "inconsistent quality" as one of the major perceptions of game meat (Hoffman *et al.*, 2004; Wassenaar *et al.*, 2019). Meat ageing is generally employed for two reasons: to improve texture i.e. tenderness; and to improve flavour (Lawrie & Ledward, 2006).

In springbok, sensory scores for attributes such as liver-like aroma and flavour, gamey flavour, metallic flavour, sour/aged flavour, off/manure flavour and tenderness have been reported to increase ($p \leq 0.05$) with ageing time (North & Hoffman, 2015). Additionally, residue scores reduced with ageing time ($p \leq 0.05$). Although an increase in positive attributes such as tenderness was reported in that study, negative attributes such as off/manure flavour also increased with ageing time. Jansen van Rensburg (1997) similarly found development of a strong unacceptable aroma with longer ageing periods (14−21 days) with 3−7 day ageing producing the '*most typical springbok flavour*'. When compared to fresh springbok, aged springbok appears to score relatively higher for attributes such as overall aroma intensity, gamey aroma and flavour, beef-like flavour, off/manure flavour, sustained juiciness and mealiness while fresh springbok scored higher for metallic aroma, liver-like aroma and flavour, sour taste and tenderness (North & Hoffman, 2015; Neethling *et al.*, 2018).

A recent study on South African consumers' attitudes towards game meat reveals that sensory attributes are one of the main factors that consumers cite in their choice to consume game meat. Additionally, attitude towards sensory characteristics was highlighted as possibly the most crucial attribute in transforming non-consumers to consumers of game meat (Wassenaar *et al.*, 2019). There is potential for the use of different ageing regimens in the optimisation of sensory characteristics in order to meet the demands of different preferences that may exist in the market. For instance in Europe, consumers appear to favour the "gamey/wild" flavour of venison while American consumers consider it a negative attribute (Wiklund *et al.*, 2003). Therefore, understanding the sensory profile of springbok aged with different techniques could inform not only processing choice for the producer but also marketing techniques for the supplier, as well as purchasing choice for the consumer.

A study on ageing of springbok meat recommended vacuum bag ageing (VAC) for a maximum of 8 days post-mortem (North & Hoffman, 2015) while another recommended 3 to 10 day skin-on (Jansen van Rensburg, 1997). The aim of this study was to assess the effect of ageing method (skin-on and VAC) and time (6, 7, 8 and 9 days) on the sensory profile of springbok *Longissimus thoracis et lumborum* (LTL) muscles and their role in improvement of the sensory profile of springbok meat. The ageing treatments were applied to LTL muscles from twenty-four springbok followed by descriptive sensory analysis (DSA) by a trained panel.

5.3 Materials and methods

5.3.1 Harvesting, slaughter and ageing

Twenty-four springbok (twelve male and twelve female) were harvested from Brakkekuil farm in Witsand, Western Cape, South Africa according to standard operating procedures (SOP/ethical approval number SU-ACUM13-00034). The adult springbok were randomly harvested at night using a light calibre rifle fitted with a suppressor with a spotlight to immobilise the animals. A headshot was used to ensure instantaneous death. The carotid and jugular blood vessels were then severed to allow carcasses to bleed out while suspended from the transport vehicle before being transported to the onsite abattoir.

At the abattoir, all carcasses were suspended by both Achilles tendons and weighed. Thereafter, the heads and hoofs were removed and the carcasses eviscerated within two hours post mortem. The carcasses were then suspended on hooks by both Achilles tendons in a cold truck and stored overnight at 0-4°C before transportation back to the meat science laboratory at the Department of Animal Science, Stellenbosch University the next day.

At the department, the carcasses were weighed and thereafter twelve (six males and six females) springbok were randomly selected for VAC ageing and the other twelve springbok were assigned to the skin-on ageing (six males and six females) for 6, 7, 8 or 9 days (Table 3.1). The former were skinned after selection and the *Longissimus thoracis et lumborum* (LTL) muscle excised (Fig. 3.1). The muscles were immediately weighed and vacuum sealed using a Multivac vacuum sealer (Model C200, Sepp Haggenmuller, Wolfertschwenden, Germany) in a vacuum bag with the following characteristics: 70 µm polyethylene and nylon; moisture vapour transfer rate of 2.2 g/m^2/24 h/1 atm, O_2 permeability of 30 cm^3/m^2/24 h/1 atm and a CO_2 permeability of 105 cm^3/m^2/24 h/1 atm. The VAC samples were then left to age for the time period allocated to each animal in a chiller at 2-4°C. At the end of the designated ageing period, the VAC samples were blotted dry, weighed and the epimysium removed. The skin-on carcasses were weighed and aged in the same cold room with a relative humidity of 79-93% for the allocated ageing time period. At the end of the ageing period, the skin-on carcasses were weighed, skinned, weighed again and the LTL muscles excised. These muscles were immediately weighed, vacuum-sealed and stored along with the VAC samples in the same cold room until physical analysis the next day.

5.3.2 Sample preparation

American Meat Science Association (AMSA) (2016) guidelines for meat cookery were followed during sample preparation. Meat samples were removed from the vacuum packaging, blotted dry with absorbent paper, weighed and the epimysium removed to allow even cooking of the muscle. Each sample was weighed, placed on a metal grid and then placed in an oven bag with a temperature probe inserted in the centre of the sample. The oven bag was then securely fastened with a twist tie to hold the temperature probe firmly in place. The samples were then placed into the industrial oven (Hobart, Paris, France) preheated to 163°C to cook to an internal temperature of 72°C. Once cooking was complete, the samples were removed from the oven bag and allowed to cool for 5 min then blotted dry and weighed to determine cooking loss (results discussed in Chapter 3).

Cooked samples were cut into 1×1×1 cm cubes and individually wrapped in aluminium foil. The meat cubes were placed in ramekins and reheated at 100°C for 7 min before serving. During testing, the cubes were kept in a 70°C water bath to maintain a constant sample temperature.

5.3.3 Descriptive sensory analysis

Descriptive sensory analysis (DSA) was done by ten trained panel members with previous experience in analysing meat samples according to AMSA guidelines (2016). Panel training was conducted using a combination of ballot and consensus methods to establish the sensory attributes characteristic to the respective meat treatments. The sensory attributes for aroma (overall intensity, gamey, beef-like, liver-like, metallic, sweet-associated sour-associated and salty), flavour (gamey, beef-like, liver-like, metallic, sweet-associated and sour-associated), and texture (initial juiciness, tenderness, sustained juiciness, sustained juiciness, mealiness, liver-like and residue) were chosen and described by the DSA panel during panel training (Table 5.1).

One of the samples in the trial was determined to be a dark firm and dry (DFD) sample owing to its uncharacteristically high pH_u, colour coordinates and appearance (Chapter 3). This sample was subjected to descriptive sensory analysis along with the rest of the samples in the trail but excluded from the statistical analysis and was discussed separately.

Table 5.1 Descriptions and scoring scale of sensory attributes for the testing panel during descriptive sensory analysis

Sensory attribute	Description	Scale
Overall aroma intensity	Intensity of aroma in first few sniffs	0=none, 100=prominent
Gamey aroma	Aroma associated with the meat from a wild animal species	0=none, 100=prominent
Beef-like aroma	Aroma associated with cooked beef fillet	0=none, 100=prominent
Liver-like aroma	Aroma associated with pan-fried beef liver	0=none, 100=prominent
Metallic aroma	Aroma associated with raw meat/blood-like	0=none, 100=prominent
Sweet-associated aroma	Aroma associated with Maillard reaction on meat surface - browning	0=none, 100=prominent
Sour-associated aroma	Aroma associated with vacuum-packed, game/wild aged meat	0=none, 100=prominent
Gamey flavour	Flavour associated with the meat from a wild animal species	0=none, 100=prominent
Beef-like flavour	Flavour associated with cooked beef fillet	0=none, 100=prominent
Liver-like flavour	Flavour associated with that of pan-fried beef liver	0=none, 100=prominent
Metallic flavour	Flavour associated with raw meat/blood-like	0=none, 100=prominent
Sour-associated taste	Taste associated with vacuum packed, game/wild aged meat	0=none, 100=prominent
Sweet-associated taste	Taste associated with Maillard reaction on meat surface - browning	0=none, 100=prominent
Salty taste	Taste associated with sodium ions	0=none, 100=prominent
Initial juiciness	Amount of fluid extruded on surface of meat sample when pressed between the thumb and forefinger (pressed perpendicular to fibres)	0=dry, 100=extremely juicy
Tenderness	Impression of tenderness after mastication	0=tough,100=extremely tender
Sustained juiciness	Amount of moisture perceived during mastication (after 5 chews)	0=dry, 100=extremely juicy
Mealiness	Disintegration of muscle fibre where mealy disintegrates into very small particles (perception within first few chews)	0=none, 100=abundant
Liver-like texture	Texture similar to that of pan-fried beef liver (spongy/pasty)	0=none, 100=prominent
Residue	Residual tissue remaining after mastication (after 10 chews) – representation of connective tissue	0=none, 100=abundant

Reference samples were used as a fixed point to facilitate scoring of attribute intensities on a line scale, thereby allowing panellists to calibrate their sensory perception during training (Table 5.2). All the reference samples were prepared in a similar manner to the test samples except for the ox liver which was pan-fried in cooking oil on medium heat for 7 min per side. For the panel training, four springbok harvested and aged (two skin-on aged and two VAC aged) for 6 days were used as references for gamey attributes

Table 5.2 Reference samples used during panel training and their associated attributes

Reference sample	Sensory attribute
Beef fillet	Beef-like aroma and flavour
	Tenderness
	Sustained juiciness
	Mealiness
	Residue
Ox liver	Liver-like aroma and flavour
	Tenderness
	Sustained juiciness
	Mealiness
	Residue
Vacuum bag aged beef	Beef-like aroma and flavour
	Tenderness
	Sustained juiciness
	Mealiness
	Residue
Dry aged beef	Beef-like aroma and flavour
	Tenderness
	Sustained juiciness
	Mealiness
	Residue
Brown meat edges	Sweet-associated aroma and flavour
Zebra meat	Beef-like aroma and flavour
	Gamey aroma and flavour
	Tenderness
	Sustained juiciness
	Mealiness
	Residue
Skin-on aged springbok	Gamey aroma and flavour
Vacuum bag aged springbok	Gamey aroma and flavour

Testing was done using a test retest method and data collected with Compusense® *five* software (Compusense, Guelph, ON, Canada). Panel members sat in individual tasting booths and were presented with four meat cubes per sample kept at ~70°C in a water bath. An unstructured line scale from 0 to 100 was used to score the samples (Table 5.1). Samples were randomly assigned to each testing sessions and labelled with three-digit random codes generated by Compusense® software. Testing was carried out in a light and temperature-controlled room (21°C). Panel members were supplied with distilled water, water biscuits and fresh apple slices (Fuji apples) as palate cleansers in between samples.

5.3.4 Instrumental tenderness

Shear force measurements (WBSF) were done on DSA samples as described in Chapter 3.

5.3.5 Statistical analysis

Statistical analysis was carried out on the means of the parameters tested using statistical software, Statistica version 13.5. Mixed model ANOVA's were conducted with judge, judge*sex, judge*ageing method and judge*ageing time as random effects. Sex was included as a fixed effect control variable. Ageing method, ageing time and ageing method*ageing time were entered as fixed effects. Degrees of freedom were calculated using the Kenward and Rogers method. For post hoc analysis, Fisher Least Significant Difference (LSD) was used. Where applicable, Pearson correlations were calculated with the physical (Chapter 3), fatty acids, volatile compounds (Chapter 4) and DSA scores in XLStat (Version 2019.1.1.56421, Addinsoft, New York USA).

5.4 Results

Across all treatments and tasting sessions, liver-like aroma, salty taste and liver-like texture were awarded the same scores by all judges. These attributes were therefore excluded from further statistical analysis. Liver-like aroma and liver-like texture was absent in 81% and 92% of the meat samples, respectively and when present were generally low (≤ 10). Salty taste was present at an intensity of 10 in 97% of the meat samples. DSA scores from the DFD sample identified in Chapter 3 were excluded from further statistical analysis and will be discussed separately.

Results for instrumental tenderness (WBSF) were reported in Chapter 3 and this chapter will explore the relationship between WBSF and the sensory textural attributes. Additionally, day 7 skin-on aged samples were noted to have uncharacteristically high WBSF values (Chapter 3) suggesting that the textural scores for these samples would differ from those of the rest of the samples.

Several interactions were reported for the DSA scores. Ageing method and time (M*T) had an effect on overall aroma intensity, gamey aroma ($p \leq 0.01$), tenderness, mealiness, residue ($p < 0.001$), metallic aroma and initial juiciness ($p < 0.05$) (Table 5.3). The scores for overall aroma intensity and gamey aroma attributes were high (> 65) and followed similar trends (Fig. 5.1). Day 6 skin-on aged samples had higher overall aroma intensity and gamey aroma scores than the day 6 VAC aged samples (Fig. 5.1). The scores for aroma attributes in skin-on samples generally decreased as the ageing days proceeded while the scores for VAC samples fluctuated with the highest scores reported on days 7 and 9 and the lowest scores generally on day 6 (Fig. 5.1). Metallic aroma scores ranging from 7.0 to 11.1 were significantly lower in day 9 skin-on samples than any other ageing day or method (Fig. 5.1). It was also observed on day 9 that the scores for the three above-mentioned aroma attributes for skin-on samples were lower than the scores for day 9 VAC samples (Fig. 5.1).

Interaction between ageing time and method also followed similar trends for tenderness ($p < 0.001$) and mealiness ($p < 0.001$) scores and a strong positive correlation was established between the two attributes ($r = 0.845$; $p < 0.001$). Scores for the two attributes ranged from (56.9 to 77.1 and 5.3 to 23.3, respectively) and days 6, 7 and 9 showed significant differences between skin-on and VAC aged samples for both attributes (Fig. 5.1). The lowest tenderness and mealiness scores were reported in day 7 skin-on samples while the highest scores were reported in day 9 skin-on samples (Fig. 5.1). With the exception of day 7, skin-on samples had higher scores for tenderness and mealiness and lower residue scores than VAC samples on the corresponding days. Residue scores (3.1 to 18.0) were significantly different between the ageing methods on days 6, 7 and 9 (Fig. 5.1). Residue was found to be negatively correlated to tenderness ($r = -0.912$, $p \leq 0.05$) and mealiness ($r = -0.774$, $p < 0.001$). Initial juiciness scores ranged from 51.6 to 58.7 and on day 7 higher in VAC samples than in skin-on samples (Fig. 5.1).

Interaction between ageing method and sex (M*S) impacted mean scores for beef-like aroma (46.1 to 48.9), metallic aroma (8.0 to 11.3), sweet-associated aroma (10.0 to 13.5) and sour-associated aroma (5.8 to 8.5) (Table 5.3). Beef-like scores were lowest ($p = 0.003$) in meat from skin-on aged female springbok samples while meat from VAC aged female springbok samples had the lowest scores ($p = 0.013$) for sour-associated aroma and highest ($p = 0.0004$) sweet-associated aroma scores (Fig. 5.2). In VAC aged samples, male springbok had higher ($p = 0.002$) metallic aroma scores than meat from female springbok (Fig. 5.2).

Interaction between ageing time and sex (T*S) had an effect on gamey aroma, beef-like aroma, sweet-associated aroma, liver-like flavour, tenderness, mealiness and residue scores (Table 5.4). On day 6, meat from female springbok had higher gamey aroma ($p = 0.011$) and sweet-associated ($p = 0.020$) scores than meat from male springbok (Fig. 5.). On days 7 and 9, meat from male springbok had higher ($p = 0.045$) beef-like aroma scores than meat from female springbok. The highest beef-like aroma and sweet associated aroma scores in meat from female springbok were recorded on day 8 while the lowest scores were on day 9 (Fig. 5.3). Liver-like aroma scores were higher ($p = 0.005$) in day 9 aged meat from male springbok than in day 7 and 8 aged samples while on day 9, meat from male springbok had higher liver-like flavour scores than meat from female springbok (Fig. 5.3). On day 7, meat from male springbok had lower ($p < 0.001$) tenderness scores than female springbok while on day 9 meat from male springbok had higher tenderness scores than female springbok on the same day (Fig. 5.3). On days 7 and 8, meat from female springbok had higher mealiness (p =

0.0004) scores than that from male springbok. Of the meat from male springbok, day 9 aged meat had the highest tenderness and mealiness scores and day 7 had the lowest scores. The highest residue scores were reported in meat from male springbok on day 7 ($p < 0.001$) (Fig. 5.3).

Ageing time had an effect on sustained juiciness (53.1 to 56.8) with day 6 having higher ($p = 0.027$) than day 7 samples (Table 5.4).

Overall means of all samples across all treatments were also obtained in order to understand the general effect of ageing on springbok LTL muscles. The overall least square means (LSM) ± standard error of the scores for the aroma attributes (overall aroma intensity, gamey, beef-like, liver-like, metallic, sweet-associated and sour-associated) of springbok LTL were 71.65 ± 0.57, 65.64 ± 0.71, 46.88 ± 0.42, 1.99 ± 0.27, 9.96 ± 0.49, 12.40 ± 0.50 and 6.97 ± 0.60, respectively. Mean scores for the flavour attributes (gamey flavour, beef-like flavour, liver-like flavour, metallic flavour, sour-associated taste, sweet-associated taste and salty taste) were 67.01 ± 0.56, 48.17 ± 0.27, 2.21 ± 0.34, 11.32 ± 0.46, 8.67 ± 0.38, 11.71 ± 0.38 and 10.04 ± 0.10, respectively. The mean scores for the texture attributes (initial juiciness, tenderness, sustained juiciness, mealiness, liver-like texture and residue) were 58.68 ± 1.15, 69.12 ± 1.42, 55.68 ± 0.82, 13.62 ± 1.40, 0.57 ± 0.17 and 6.83 ± 1.32, respectively. As per Chapter 3, mean WBSF was 32.36 ± 15.79 N

The DFD sample received higher scores than the overall mean scores (LSM ± s.e.) for some of the positive attributes such as tenderness (73.60 ± 3.97), sustained juiciness (61.95 ± 3.24) and lower scores for some negative attributes such as mealiness (9.75 ± 3.99) and residue (4.50 ± 2.17). However, the samples did score higher in some negative attributes such as metallic aroma (13.80 ± 2.16), liver-like aroma, flavour and texture (5.10± 2.26, 10.65 ± 2.21 and 4.00 ± 1.63, respectively) and lower for some positive attributes such as initial juiciness (50.35 ± 3.00).

Table 5.3 p-values for the effects of ageing method, time and sex and their interactions on sensory attributes of springbok *Longissimus thoracis et lumborum m*uscle.

Attribute	Ageing method	Time	Sex	M*T	M*S	S*T
Overall aroma intensity	0.077	0.053	0.696	**0.004**	0.780	0.114
Gamey aroma	0.159	0.095	0.798	**0.001**	0.310	**0.011**
Beef-like aroma	**0.001**	**0.044**	0.068	0.904	**0.003**	**0.045**
Metallic aroma	0.919	0.222	**0.032**	**0.019**	**0.002**	0.370
Sweet-associated aroma	0.251	0.075	0.133	0.917	**0.000**	**0.020**
Sour-associated aroma	**0.048**	0.331	0.109	0.154	**0.013**	0.345
Gamey flavour	0.779	0.426	0.107	0.364	0.591	0.096
Beef-like flavour	0.767	0.900	0.760	0.507	0.998	0.149
Liver-like flavour	0.753	**0.042**	0.910	0.864	0.452	**0.005**
Metallic flavour	0.191	0.377	0.233	0.296	0.135	0.217
Sour-associated taste	0.921	0.113	0.649	0.698	0.593	0.129
Sweet-associated taste	0.341	0.492	0.135	0.236	0.654	0.543
Initial juiciness	0.340	0.297	0.984	**0.045**	0.064	0.064
Tenderness	0.371	**0.000**	**0.028**	**0.000**	0.524	**0.000**
Sustained juiciness	0.126	**0.027**	0.722	0.476	0.510	0.968
Mealiness	**0.044**	**0.001**	0.163	**0.000**	0.103	**0.000**
Residue	0.484	**0.000**	0.092	**0.000**	0.380	**0.000**

Table 5.4 Effects of ageing method, time and sex on LS means ± standard errors of the DSA scores of springbok LTL muscle

Attribute	Ageing method		Ageing time (days)				Sex	
	Skin-on	**VAC**	**6**	**7**	**8**	**9**	**Male**	**Female**
Overall aroma intensity	72.3 ± 1.29	70.6 ± 1.35	70.7 ± 1.38	73.6 ± 1.43	70.5 ± 1.63	71.2 ± 1.40	71.3 ± 1.29	71.6 ± 1.31
Gamey aroma	66.1 ± 1.42	64.6 ± 1.50	65.2 ± 1.54	67.6 ± 1.61	64.0 ± 1.85	64.5 ± 1.56	65.2 ± 1.43	65.5 ± 1.45
Beef-like aroma	45.7^{y} ± 0.82	48.5^{x} ± 0.88	46.9^{b} ± 0.91	47.1^{ab} ± 0.96	48.8^{a} ± 1.14	45.6^{b} ± 0.93	47.8 ± 0.83	46.4 ± 0.85
Metallic aroma	9.7 ± 1.41	9.8 ± 1.45	10.2 ± 1.47	10.9 ± 1.50	8.9 ± 1.65	8.9 ± 1.48	10.6^{b} ± 1.41	8.9^{a} ± 1.42
Sweet-associated aroma	12.0 ± 0.88	13.0 ± 0.94	11.3 ± 0.98	12.5 ± 1.03	14.5 ± 1.24	11.7 ± 1.00	11.9 ± 0.87	13.1 ± 0.90
Sour-associated aroma	7.8^{a} ± 1.57	6.0^{b} ± 1.61	6.6 ± 1.70	8.1 ± 1.74	5.4 ± 1.88	7.3 ± 1.72	7.5 ± 1.57	6.2 ± 1.58
Gamey flavour	66.9 ± 1.98	67.3 ± 2.03	65.9 ± 2.01	68.1 ± 2.05	67.2 ± 2.22	67.1 ± 2.03	66.3 ± 1.92	67.9 ± 1.94
Beef-like flavour	48.0 ± 0.81	47.8 ± 0.86	48.3 ± 0.96	47.8 ± 0.99	47.4 ± 1.15	48.0 ± 0.97	47.8 ± 0.82	48.0 ± 0.84
Liver-like flavour	2.0 ± 0.67	2.2 ± 0.72	2.8^{a} ± 0.75	0.8^{b} ± 0.79	2.0^{ab} ± 0.95	2.8^{a} ± 0.77	2.1 ± 0.69	2.1 ± 0.71
Metallic flavour	10.5 ± 1.39	12.5 ± 1.44	11.2 ± 1.36	12.6 ± 1.40	11.4 ± 1.58	10.6 ± 1.37	11.9 ± 1.27	11.0 ± 1.28
Sour-associated taste	8.6 ± 1.25	8.7 ± 1.29	7.9 ± 1.37	10.2 ± 1.41	7.2 ± 1.56	9.3 ± 1.39	8.8 ± 1.22	8.5 ± 1.24
Sweet-associated taste	11.8 ± 1.03	11.0 ± 1.08	10.7 ± 1.14	11.8 ± 1.18	11.0 ± 1.34	12.1 ± 1.15	10.9 ± 1.02	12.0 ± 1.03
Initial juiciness	57.3 ± 1.80	59.1 ± 1.96	59.2 ± 2.19	55.1 ± 2.31	58.6 ± 2.79	59.9 ± 2.23	58.2 ± 1.89	58.2 ± 1.95
Tenderness	69.3 ± 2.23	67.9 ± 2.32	69.1^{x} ± 2.37	62.3^{y} ± 2.44	70.3^{x} ± 2.73	72.7^{x} ± 2.39	66.9^{b} ± 2.24	70.3^{a} ± 2.27
Sustained juiciness	54.5 ± 2.29	56.6 ± 2.35	58.4^{a} ± 2.46	52.7^{b} ± 2.52	56.5^{ab} ± 2.75	54.8^{ab} ± 2.48	55.4 ± 2.29	55.8 ± 2.31
Mealiness	15.0^{a} ± 2.40	11.4^{b} ± 2.48	11.4^{yz} ± 2.60	8.4^{z} ± 2.66	15.1^{xy} ± 2.94	18.0^{x} ± 2.62	12.0 ± 2.43	14.5 ± 2.46
Residue	7.3 ± 1.74	8.4 ± 1.82	6.6^{y} ± 1.84	13.0^{x} ± 1.91	5.8^{y} ± 2.20	5.9^{y} ± 1.86	9.4 ± 1.81	6.3 ± 1.84

^{a,b} means in the same row (within the main effect) with different superscripts differ significantly from each other p ≤ 0.05

^{x,y} means in the same row (within the main effect) with different superscripts differ significantly from each other p ≤ 0.01

DSA = Descriptive sensory analysis; LTL = *Longissimus thoracis at lumborum*

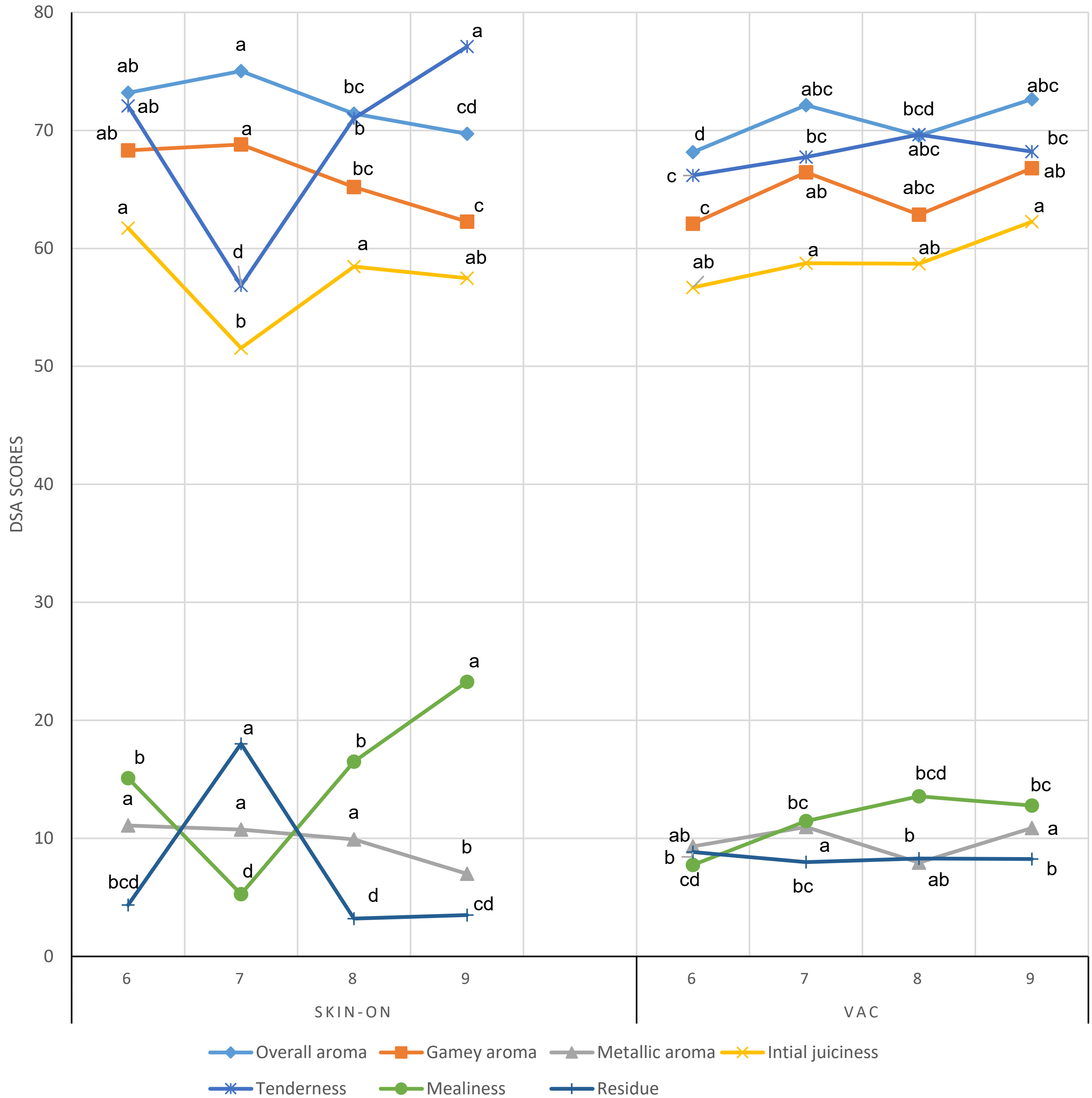

Figure 5.1 Interactions between ageing method and time on the LSM DSA scores for selected aroma and texture attributes of springbok *Longissimus thoracis et lumborum* muscles. [a-d] Means with different superscripts differ significantly from each other.

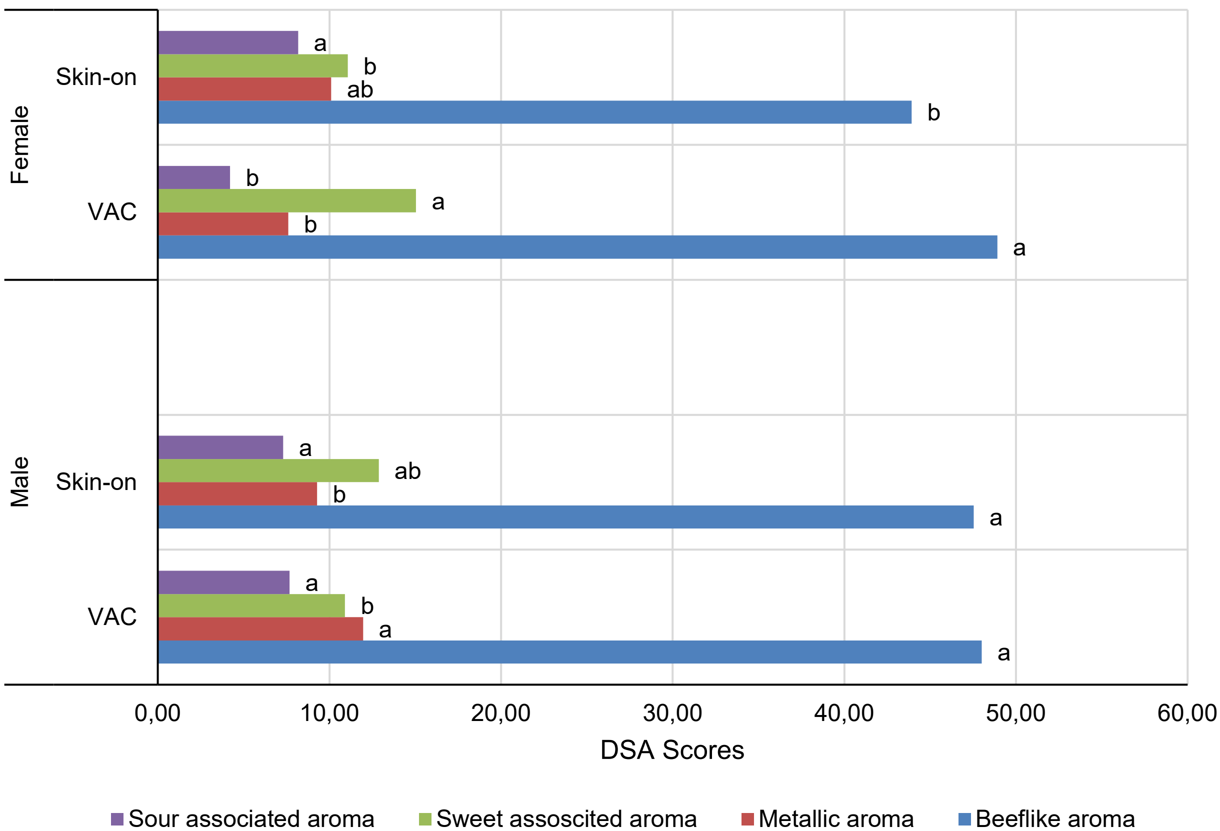

Figure 5.2 Interaction between ageing method and sex LSM scores for selected aroma attributes with significant differences of springbok *Longissimus thoracis et lumborum* muscle

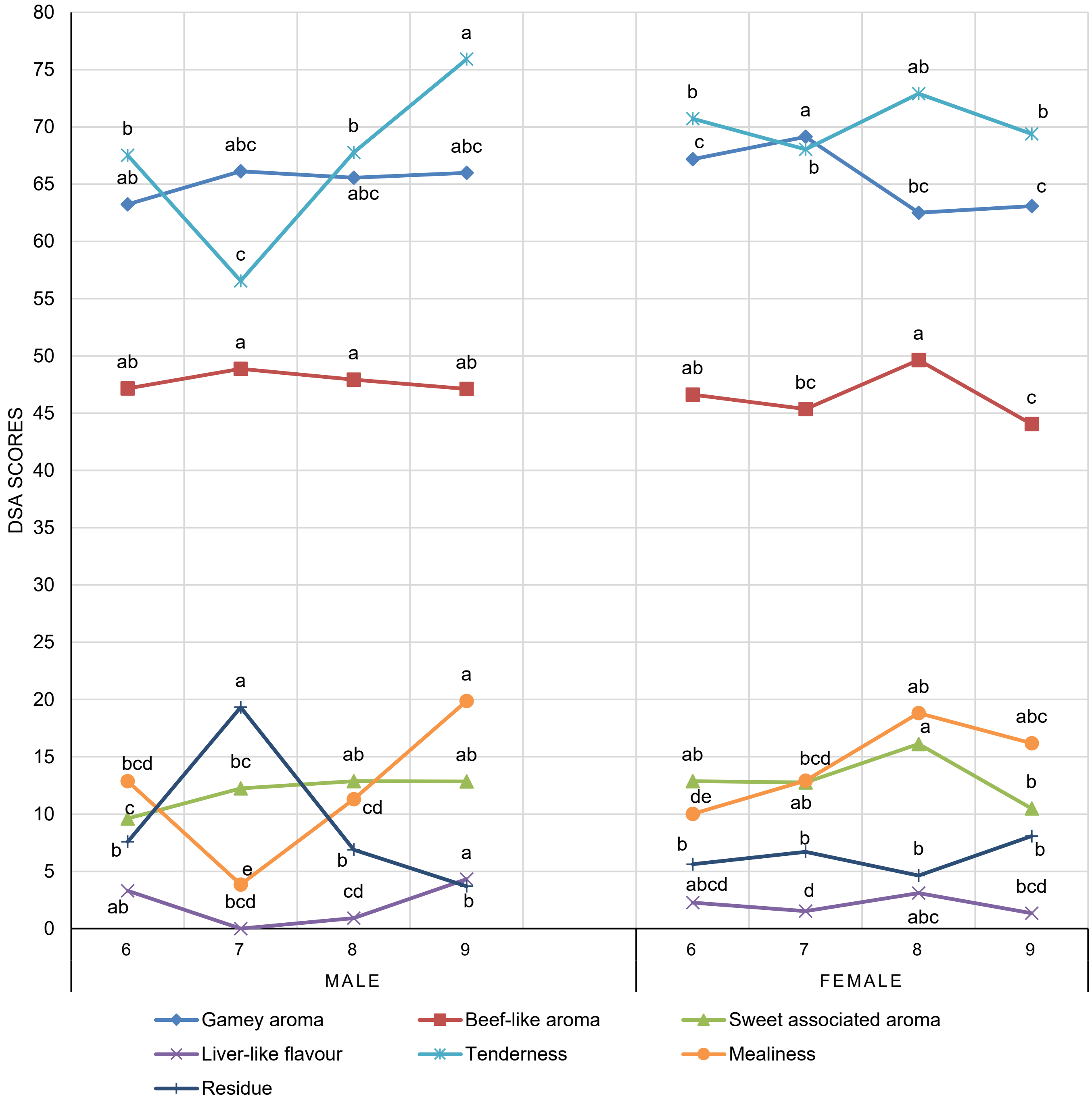

Figure 5.3 Interaction between ageing time (days) and sex LSM scores for selected sensory attributes of springbok *Longisimuss thoracis et lumborum* muscle

5.5 Discussion

The significant correlation established in the current study between gamey aroma and overall aroma intensity (Fig. 5.1) (r = 0.892, p < 0.001) suggests that as with previous studies on springbok and other game meat (North & Hoffman, 2015; Neethling, 2016), gamey aroma is the largest contributor to the overall aroma profile springbok meat. Moderate to strong significant correlations between gamey aroma and other aroma attributes established in the current study (liver-like aroma; $r = 0.620$ & $p = 0.002$, metallic aroma; $r = 0.687$ & $p < 0.001$ and sour-associated aroma; $r = 0.807$ & $p < 0.001$) similarly suggest that these aroma attributes contribute to the perception of gamey aroma in aged springbok meat.

While significant differences in scores for overall aroma intensity and gamey aroma between day 6 and 7 VAC aged samples were found (Fig 5.1), North and Hoffman (2015) found no differences in scores for gamey aroma and overall aroma intensity ($p = 0.509$ and $p = 0.202$, respectively) in their study on VAC aged springbok LTL muscles. They also reported a significant increase in metallic aroma ($p = 0.004$) between 3 and 8 days of ageing which was not observed in the VAC samples in current study (Fig. 5.1). This highlights the challenge found in directly comparing the trends observed with ageing time between the two studies. While the current study used LTL muscles from different animals for each ageing day, North and Hoffman (2015) used LTL muscles from the same animals for the duration of each ageing period and this could be a reason for the dissimilar trends observed.

The overall game flavour intensity has been associated with PUFA content, specifically α-linolenic acid (C18:3n-3) content, in springbok meat ($r = 0.47$, $p < 0.05$) (Hoffman *et al.*, 2007). Similarly, in meat from other game species it was suggested that the gamey/wild flavour associated with game arises from compounds associated with long chain omega-3 (n-3) fatty acids obtained through grazing (Miller *et al.*, 1986; Swanson & Penfield, 1991). However, in the current study only weak correlations were established between gamey aroma and flavour and total n-3 content and ($r = -0.223$; $p = 0.294$ and $r = -0.196$; $p = 0.359$, respectively) as well as percentage linoleic acid ($r = -0.054$; $p = 0.804$ and $r = 0.177$; $p = 0.409$, respectively) (Chapter 4). Neither strong nor significant correlations between the volatile compounds previously reported to strongly correlate with gamey aroma and flavour (Neethling, 2016) were observed in the current study. Of the volatile compounds identified in the current study (Chapter 4), significant albeit weak correlations with gamey aroma were only established for 1-octanol ($r = -0.456$; $p = 0.025$), methyl decanoate ($r = 0.478$; $p = 0.018$) and caprolactam ($r = 0.44$; $p = 0.032$). Gamey flavour scores were only significantly correlated with 2-pentanol ($r = 0.553$; $p = 0.005$), D-limonene ($r = 0.466$; $p = 0.022$), methyl octanoate ($r = 0.479$; $p = 0.018$) and pentadecanoic acid ($r = -0.436$; $p = 0.033$). The weak nature of the correlations established in the current study as well as in previous studies (Neethling, 2016) coupled with the complex nature of flavour suggests that further investigation is needed to isolate the source of gamey flavour attributes in springbok meat.

The higher scores for overall aroma intensity and gamey aroma in skin-on aged samples on day 6 than VAC aged samples (Fig. 5.1) suggest that skin-on aged samples has a stronger gamey flavour. In red deer (*Cervus elaphus*), skin-on ageing at 10°C for at least 24 h was similarly found to significantly increase flavour specific to venison as well as gamey and sweet aromas (Soriano *et al.*, 2016). Swanson and Penfield (1991) suggested that a mild gamey flavour in reindeer would appeal to consumers who do not typically consume exotic meats. Similarly, Wiklund *et al.* (2003) noted a preference in European consumers for “wild/gamey” flavour in venison while in other countries such as the USA this attribute was considered negative. In South Africa, restaurants surveyed in the Eastern Cape indicated that they perceived their customers as not liking the gamey flavour of game meat (Radder, 2002). The significant difference in overall aroma intensity and gamey aroma scores between VAC and skin-on samples on day 6 (Fig. 5.1) suggests that VAC ageing for 6 days could potentially be implemented in marketing towards consumers that prefer meat with a milder gamey flavour. For such consumers, 6 day VAC aged springbok meat could be marketed whereas consumers that prefer a stronger gamey flavour can opt for the skin-on aged meat.

While there may be varying consumer perceptions on gamey associated attributes such as gamey and liver-like flavour in game meat, it is important to note that these attributes are inherent to the species as shown by Rødbotten *et al.* (2004). Although strong gamey flavour has previously been linked to poor harvesting, processing and preparation techniques (Talbot, 1965), under optimal conditions these attributes still characterise the flavour profile of game meat (Rødbotten *et al.*, 2004; Hoffman *et al.*, 2007; North & Hoffman, 2015; Neethling *et al.*, 2018). Additionally, the strong correlations established between gamey aroma and liver-like aroma, metallic aroma and sour-associated aroma in this study further indicate that these attributes characterise aged springbok meat as well. Focus during quality assessment of springbok meat should therefore be placed on maintaining a quality consistent with game and not necessarily on achieving a flavour profile similar to domesticated species.

Metallic aroma was moderately negatively correlated to three C8 alcohols i.e. 1-octanol ($r = -0.703$; $p = 0.0001$), 2-octen-1-ol ($r = -0.608$; $p = 0.002$) and 1-octen-3-ol ($r = -0.611$; $p = 0.002$) that were present in twelve, nineteen and twenty-four samples respectively (Chapter 4). Methyl hexanoate and furan 2-pentyl ($n = 24$ and 15, respectively) were also negatively correlated to metallic aroma ($r = -0.559$; $p = 0.005$ and $r = -0.434$; $p = 0.034$, respectively). Of the volatile compounds reported above, furan 2-pentyl and 1-octen-3-ol have previously been described in literature as having a metallic aroma in beef stew (Peterson & Chang, 1982; Susan Brewer, 2018). It is therefore unclear why these compounds would be negatively correlated to metallic aroma and unclear whether the correlations reported are relevant. As with gamey aroma, further studies would be required in order to better understand the source of metallic flavour in springbok meat.

Sex interacted with both ageing method and ageing time resulting in significant differences in most of the aroma attributes as well as some of the texture attributes (Figs. 5.2 & 5.3). Considering the amount of variation due to sex that was detected in the fatty acids profile in Chapter 4, it can be expected that aroma attributes would also differ due to sex as fatty acids are known to considerably contribute to aroma profile (Wood *et al.*, 2003). Although no particular fatty acid could be linked to variation in aroma profile in the current study, fatty acids and their oxidative products have long been thought to be the source of many aroma attributes of meat (Wood *et al.*, 2003; Lawrie & Ledward, 2006). When considering ageing method and sex (M*S) interactions, sweet associated aroma is highest ($p < 0.001$) in VAC aged meat from female springbok which also had the lowest ($p = 0.013$ and $p = 0.002$, respectively) sour associated aroma and metallic aroma scores (Fig. 5.2). Metallic aroma was positively correlated to sour associated aroma ($r = 0.692$; $p = 0.0002$) while sweet associated aroma negatively correlated with both metallic and sour associated aroma ($r = -0.482$; $p = 0.017$ and $r = -0.654$; $p = 0.001$, respectively). In fresh meat from various game species, the only similar significant correlation reported was between sour and metallic aroma ($r = 0.583$; $p = 0.047$) (Neethling, 2016). Although these reported correlations suggest that that perception of these attributes in aged springbok meat could be linked, this would have to be confirmed in further studies with less variables and greater sample sizes.

All textural attributes with the exception of sustained juiciness were impacted by ageing method and time (Fig. 5.1). Of the textural attributes, tenderness and residue scores were found to positively correlate with WBSF ($r = -0.802$; $p < 0.0001$ and $r = 0.741$; $p < 0.0001$, respectively) while sustained juiciness and mealiness only exhibited moderate correlations with WBSF ($r = -0.570$; $p = 0.004$ and $r = -0.581$; $p = 0.004$, respectively). Tenderness scores were also strongly correlated to mealiness and residue scores ($r = 0.845$; $p < 0.0001$ and $r = -0.912$; $p < 0.0001$, respectively).

The relationship between tenderness, residue, mealiness scores and WBSF can be explained by the enzymatic myofibrillar degradation that occurs during ageing (North, 2014; Matarneh *et al.*, 2017). Breakdown of muscle fibres results in shorter muscles fibres that produce finer particles on chewing (North, 2014). Shorter muscle fibres shear easier thus resulting in lower WBSF recorded and the finer particles produced on chewing result in increased perception of mealiness while leaving little residual particles after chewing (Davey & Gilbert, 1969; North, 2014). For example, in DFD samples, high tenderness scores have been attributed to shorter sarcomeres with greater moisture content leading to less extracellular spaces around muscle fibres (Tornberg, 1996; Matarneh *et al.*, 2017). Thus, differences observed between skin-on and VAC aged samples (Fig. 5.1) can be attributed to greater myofibrillar degradation in skin-on aged samples with ageing time than in VAC aged samples. Muscle stretching is a common method of improving meat tenderness typically done by carcass suspension before and during rigor mortis (Hopkins, 2017). Suspension of carcasses against gravity resulting in a pulling force experienced throughout the carcass during skin-on ageing could facilitate further myofibrillar breakdown producing more tender meat with greater mealiness scores and less residue observed with skin-on aged meat (Fig. 5.1). This however would have to be confirmed in further studies on the impact of the two ageing methods on the myofibrillar degradation of springbok meat.

The perception of juiciness and tenderness in meat by consumers is attributed to moisture present in meat (Campo *et al.*, 1999; Warner, 2017), as well as intramuscular fat content (IMF) (Thompson, 2004; Corbin *et al.*, 2015; Frank *et al.*, 2016). The correlations between IMF content and juiciness attributes in this study were weak and not significant (initial juiciness $r = 0.018$; $p = 0.936$ and sustained juiciness $r = 0.225$; $p = 0.303$). Additionally, no strong correlations were established between initial juiciness and the other textural attributes but significant moderate correlations with sustained juiciness and percent cooking loss (Chapter 3) were established ($r = 0.533$; $p = 0.009$ and $r = -0.427$; $p = 0.042$, respectively). Lagerstedt *et al*. (2008) suggested that higher juiciness levels in aged then chilled beef steaks compared to aged then frozen beef steaks caused sensory panellist to score the former highly for tenderness. This phenomenon whereby perception of one attribute influences perception of another is described as the “halo effect” (Roeber *et al.*, 2000; Corbin *et al.*, 2015; Miller, 2017). The lower tenderness scores for day 7 skin-on aged samples could the reason these samples were also perceived as less juicy (Fig. 5.1). However as the difference in scores was less than ten, these changes can be interpreted as minor (North & Hoffman, 2015).

Tenderness is a vital positive textural attribute and its close relationship with mealiness and residue should be considered when optimising ageing time and method in springbok meat. Higher mean tenderness scores (76.1 ± 2.17) as well as lower mean residue (2.9 ± 0.59) and mealiness (8.2 ± 1.18) scores have been reported in fresh springbok meat (Neethling *et al.*, 2018) while similar scores to those in the current study were found in VAC aged springbok (North & Hoffman, 2015). Although a major benefit of ageing is the tenderisation achieved (Lawrie & Ledward, 2006), fresh springbok meat is already tender (Hoffman *et al.*, 2007; Neethling *et al.*, 2018) therefore the major benefit of ageing springbok meat would be in improvement of aroma and flavour attributes.

With regard to the mean DSA scores, liver-like aroma and flavour scores across all treatments were lower than five (1.99 ± 0.27 and 2.21 ± 0.34, respectively) signifying the low intensity of these attributes. Aged springbok has previously been reported to have similarly lower scores for liver-like aroma and flavour (1.9 ± 0.42 to 2.7 ± 0.28 and 1.2 ± 0.30 to 3.6 ± 0.55, respectively) when compared to fresh springbok (17.7 ± 0.79 and 13.2 ± 1.05, respectively) (North & Hoffman, 2015; Neethling *et al.*, 2018). This clearly highlights the importance of ageing in flavour improvement with regards to lowering of unpleasant attributes. Liver-like flavour was the only flavour attribute that had scores affected by any of the treatments with the highest score, 4.31 ± 0.94, recorded in 9 day aged male samples ($p = 0.005$). This was still far below the scores recorded for fresh springbok (Neethling *et al.*, 2018). The low scores for the liver-like attributes suggests that these attributes have a low intensity and do not contribute greatly to the flavour profile of aged springbok meat.

Sour associated aroma and taste was of particular interest in this study due to the employment of VAC ageing. North and Hoffman (2015) reported an increase in scores for both attributes ($p = 0.004$ and $p = 0.003$, respectively) with ageing time possibly linked to production of lactic acid as a result growth of lactic acid bacteria (LAB). Li *et al.* (2014) found that VAC aged beef had higher sour taste scores ($p = 0.013$) as well as LAB counts ($p = 0.001$) than dry aged and special bag aged samples after 8 days of ageing. In the current study, VAC aged samples had lower sour-associated aroma scores than skin-on samples (Table 5.4) and no difference between sour-associated taste was reported ($p = 0.921$) between the two methods. When considering the lactic acid production from LAB growth as a source of sour aroma and flavour, higher LAB growth in VAC samples ($p = 0.102$) should translate in higher scores for sour attributes in VAC samples. However, from the current findings, it is therefore unlikely that LAB growth was solely responsible for the sour attributes observed in aged springbok meat in the current study. Other possible sources of sour attributes listed in literature include organic acids and free amino acids (Elmore & Mottram, 2009) that were not analysed in the current study. However, as with the previous study on VAC aged springbok (North & Hoffman, 2015), the scores for sour-associated attributes were low on the 100-point scale and can be considered to not have a major impact on the springbok flavour profile.

Flavour attributes in the current study were largely unaffected by the ageing treatments implemented (Table 5.3). When aged up to 8 days, few significant changes in flavour attributes were found in springbok meat (North & Hoffman, 2015). Similarly, flavour scores for springbok meat aged up to 10 days did not differ significantly ($p < 0.001$) regardless of ageing method implemented (Jansen van Rensburg, 1997). This suggests that ageing times previously suggested for springbok meat (Jansen van Rensburg, 1997; North & Hoffman, 2015) are indeed capable of producing meat with consistent flavour attributes.

One of the chief deterrents to consistent purchasing and consumption of game meat cited by consumers is inconsistent meat quality (Hoffman *et al.*, 2004). The trend observed over time for aroma attributes appears to be more stable for skin-on samples than VAC samples (Fig. 5.1). With regard to textual attributes, VAC samples displayed a more stable predictable trend of increasing tenderness than skin-on aged samples (Fig. 5.1). However, it is important to note that on day 7 skin-on samples had abnormally high WBSF values, possibly as a result of inter-animal variation such as age or ante-mortem stress levels (Chapter 3) and can be considered as the exception and not the norm. Therefore, if day 7 skin-on aged samples in the current study are considered as outliers, a stable trend could also be expected for the textural attributes of skin-on sample. Skin-on ageing as a method could possibly produce more consistent quality meat than VAC ageing but this would have to be confirmed in future research where the same muscle from the same animal is subjected to both ageing treatments over a period of time in order to eliminate any inter-animal effects.

Despite the fact that the sensory profile of the DFD meat sample did not differ greatly from that of normal meat pH, processing and sale of DFD meat is another point at which game meat of inconsistent quality can be introduced into the market. Although the DFD sample had higher mean scores for some positive attributes (tenderness and sustained juiciness) and lower scores for negative attributes (metallic aroma and liver-like attributes), its uncharacteristic dark and firm appearance (Faustman & Suman, 2017) and shorter shelf-life (Shange *et al.*, 2019), it is not recommended that DFD meat be processed along with normal meat.

5.6 Conclusion

While several differences between descriptive sensory analysis (DSA) scores as a result of the treatments applied were noted, the complex nature of flavour as well as the number of treatments applied made it hard to draw definitive conclusions on the source of some of the variations observed. Nonetheless, this study did still manage to provide clear insight into some of the effects of ageing on the sensory profile of springbok meat. Scores for liver-like attributes were found to be lower in aged springbok meat than fresh springbok meat suggesting the value in ageing as a method of reducing occurrence negative sensory attributes. Sour-associated attributes in aged springbok loins in that had been thought to arise from growth of LAB could not be correlated to LAB growth previously reported (Chapter 3) in the current study.

Furthermore, differences in overall aroma intensity and gamey aroma scores between skin-on and VAC ageing on day 6 suggest that the two ageing treatments can be utilised to service different niches in the consumer base. Six day VAC aged springbok meat could be marketed to consumers who prefer meat with mild gamey attributes while the skin-on aged meat can be marketed towards consumers who prefer meat with stronger gamey attributes. Finally, there is a need to understand consumer preferences when it comes to game meat quality in order to accurately implement the ageing treatments explored in the current study.

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CHAPTER 6

6.1 General discussion

It is clear that the health benefits of game meat consumption (Hoffman *et al.*, 2007a) as well as environmentally sustainable means of production (Hoffman, 2007) appeal to consumers of game meat (Hoffman *et al.*, 2005; Wassenaar *et al.*, 2019). The environmentally friendly extensive production system of game animals in South Africa ensures the maintenance of the favourable nutrient profile in game meat desired by game meat consumers (Wiklund *et al.*, 2001; Hoffman *et al.*, 2003; Hoffman, 2007). Standardisation of practices across the game meat production industry is therefore essential in providing consumers with products of consistent quality.

Ageing has long been recommended as a way of improving texture and flavour attributes of game meat (Skinner, 1996). However, springbok meat has clearly been shown to be tender even without ageing, therefore major gains made from ageing are in flavour improvement (Hoffman *et al.*, 2007b; North & Hoffman, 2015; Neethling *et al.*, 2018). Improved scores for sensory attributes such as tenderness, residue, and gamey flavour and overall flavour with ageing has previously been reported for springbok meat (Jansen van Rensburg, 1997; North & Hoffman, 2015). Thus considering the ageing treatments previously recommended for springbok meat (Jansen van Rensburg, 1997; North & Hoffman, 2015), the aim of this study was therefore to assess the impact of the two ageing methods (skin-on and VAC) and ageing time (6 to 9 days) on various meat quality attributes of springbok LTL muscles.

From the carcass characteristics examined in this study (Chapter 3), it was found that skin-on aged carcasses had lower dressing percentages than carcasses where LTL muscles were VAC aged. This was postulated to be as a result of moisture loss from exposed carcass surfaces during skin-on ageing. As carcasses were washed before skinning, it is possible that most of the moisture lost from the skin originated from this process. As carcasses are typically priced on a weight basis (ZAR/kg) the results show that if skin-on carcasses are sold, they would fetch a lower price. However, since moisture loss was not on the prime cuts of these carcasses, few differences in weight between skin-on and VAC aged muscles would be expected. However, further analysis of moisture loss from springbok carcasses during skin-on ageing and its impact on income generated would be needed to determine if indeed majority of moisture loss observed is as a result of washing carcasses.

Concerns of the impact of ageing treatments on meat safety and nutritional quality were addressed by analysing the microbial quality (Chapter 3) and fatty acid profile (Chapter 4). Ageing time had a slight impact on APC counts as previously observed (Newsome *et al.*, 1984; Buys *et* *al.*, 1997; Hulánková *et al.*, 2018) with 9 ageing days resulting in slightly higher ($p = 0.094$) APCs than day 6. However, across ageing treatments and times, APCs were well below the European Union (EU) standard set for meat for human consumption (EC No 2073/2005, 2005). LAB appeared to be more prevalent in VAC aged samples with slightly higher counts ($p = 0.102$) than in skin-on aged samples. The favourability of the anaerobic environment to LAB growth has previously been reported (Kerry & Tyuftin, 2017) and the subsequent LAB growth has been shown to negatively impact shelf-life of meat samples (Nortjé & Shaw, 1989).

Sex was the main factor that influenced the fatty acid profile (Chapter 4) likely as a result of differing IMF content between male and female springbok (Wood *et al.*, 2008; Clausen *et al.*, 2009; Resconi *et al.*, 2013; López-Bote, 2017; Wood, 2017). Across all treatments, the important ratios (PUFA:SFA and n-3:n-6 PUFA) were within recommended specification for a healthy diet (Wood *et al.*, 2003; Simopoulos, 2004; Schmid, 2011). Recommended ageing methods and times did not negatively affect the fatty acid profile of springbok meat thus aged meat can be confidently marketed as providing similar health benefits as fresh springbok meat.

This study also explored the impact of ageing on the volatile compounds produced once aged springbok meat was cooked (Chapter 4). The results showed an abundance of compounds linked to lipid oxidation that likely arose from the various PUFAs present in springbok meat (Whitfield, 1992; Resconi *et al.*, 2013; López-Bote, 2017). Ageing resulted in an increase in the number of volatile compounds produced as reported in previous ageing studies (Watanabe *et al.*, 2015; Maggiolino *et al.*, 2018). As no strong correlations between the volatile compounds detected and sensory scores for aroma attributes were established, it was difficult to link the volatile compound profile to the sensory profile in this study (Chapter 5). Further studies with a larger sample size aimed at confirmation and quantification of the identified volatile compounds as well as application of gas chromatographic analysis coupled with olfactometric detection (GC-O) is recommended to better understand the relationship between the compounds detected and the aroma profile experienced (Brattoli *et al.*, 2013). Results obtained in this and previous studies (Neethling, 2016) can serve as foundations for confirming volatile compounds and relationships observed. Since no stark differences were identified between the ageing treatments applied, this suggests that neither ageing time nor method affected the volatile compounds profile nor by extension, the aroma profile of the aged meat.

However, there were notable influences of ageing method and time on sensory scores for some aroma attributes (overall aroma intensity, gamey, beef-like, and metallic aromas) as assessed during descriptive sensory analysis (Chapter 5). Stronger gamey characteristics such as gamey and metallic attributes (Rødbotten *et al.*, 2004) appeared to be linked with skin-on ageing. This knowledge can be used in marketing with skin-on aged meat appealing to markets in which strong gamey characteristics are considered desirable (Swanson & Penfield, 1991; Wiklund *et al.*, 2003). There is potential to cater for non-game meat consumers looking to make the shift but are hindered by the sensory characteristics of game meat (Wassenaar *et al.*, 2019). For such consumer markets, 6 day VAC aged springbok meat can be marketed due to its weaker gamey attributes.

High tenderness scores (> 65) reported across all treatments would appeal to meat consumers who have been shown to generally prefer tender meat (Huffman *et al.*, 1996; Hoffman *et al.*, 2004). It appears that skin-on samples scored higher for tenderness although VAC samples were also suitably tender. Additionally, lower scores were observed for negative sensory attributes such as liver-like attributes (aroma; 1.99 ± 0.27 and flavour; 2.21 ± 0.34) in aged meat in this study than in fresh springbok meat (17.7 ± 0.79 and 13.2 ± 1.05, respectively) from a previous study (Neethling *et al.*, 2018) indicating that ageing did improve the flavour profile of springbok meat.

Several studies have been carried out with trained sensory panels on meat derived from various South African game species (Hoffman *et al.*, 2007, 2009, 2010; North & Hoffman, 2015; Neethling, 2016; Needham *et al.*, 2019). These trials have served to build an understanding of the flavour profile of game meat and how it varies as a result of different factors such as species, sex and region of production. In the same timeframe, only two large scale consumer analyses on game meat were done in 2002 and then more recently in 2016 (Crafford, 2002; Wassenaar, 2016). In both cases, surveys were employed to understand consumers' attitudes towards consumption of game meat. Consumer attitudes towards game meat consumption as well as general purchasing trends have changed over the years. For example while consumers appeared to be indecisive regarding the ethics of culling game animals and unaware of the benefits of game meat (Hoffman *et al.*, 2005), in a more recent study consumers rated health benefits and game production ethics positively and considered these among the most important attributes informing their decisions to consume game meat (Wassenaar *et al.*, 2019).

It then became exceedingly clear during this study that although consumers' attitudes towards game meat in South Africa are well understood (Hoffman *et al.*, 2005; Wassenaar *et al.*, 2019), consumer preference data is lacking. The fundamental difference between domesticated red meat species and game (Rødbotten *et al.*, 2004) further suggests that preferences for the former cannot be completely relied upon to inform preferences for the latter (Wassenaar *et al.*, 2019). Therefore, there is a need to assess consumers' preferences in order to inform how springbok and game meat as a whole can be processed and marketed.

The animal sample size utilised in the current study was not suited to the experimental outlay. When investigating first order interactions between the main treatments (ageing method, ageing time and sex), this resulted in lower than ideal sample sizes (Fig. 3.1). However as repeatedly demonstrated in previous research (Jansen van Rensburg, 1997; North & Hoffman, 2015; Needham *et al.*, 2020) as well as the current study, the effects of sex and ageing time on particular meat quality attributes could not be ignored. This, in addition to the tightly coupled nature of meat quality attributes (Matarneh *et al.*, 2017; Miller, 2017), made it necessary to consider both ageing time and sex as main factors along with ageing method in the current study. The significant interactions observed in the current study can therefore be investigated further with larger sample sizes of at least six animals per treatment, where possible.

Overall, there were no major differences in meat quality observed as a result of the ageing methods applied; thus, no single ageing treatment stood out as the definitive choice. This implies that no severe differences in meat quality can be expected when ageing treatments are applied. However, since game meat is perceived as having inconsistent quality by consumers (Hoffman *et al.*, 2005), once an ageing treatment has been chosen, it should be adhered to in order to produce meat of consistent quality.

6.2 References

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Chapter 7

Addendums

7.1 Addendum A

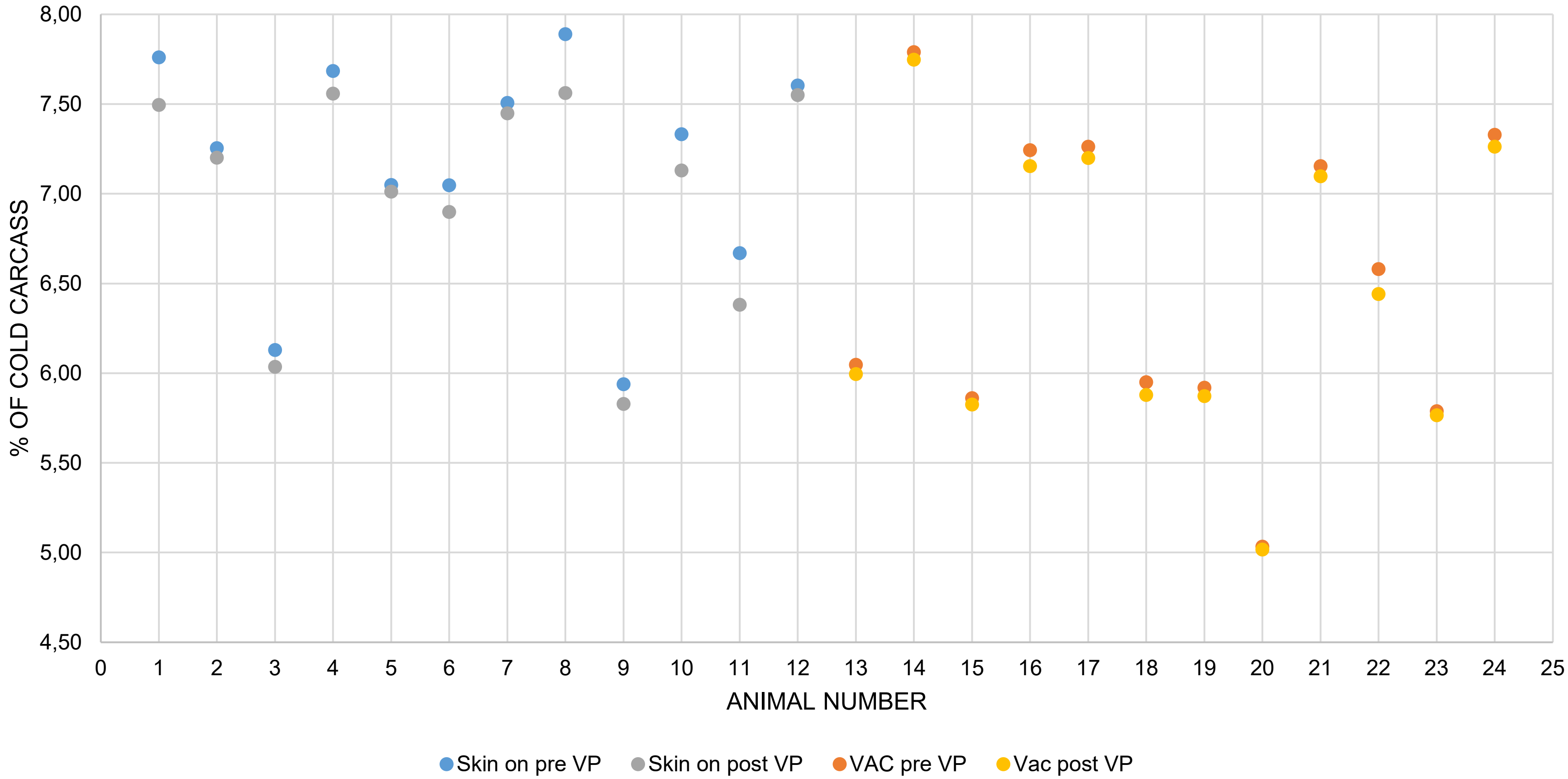

Figure A. Weight of each animal's *Longissimus thoracis et lumborum* muscle (LTL) muscle before and after vacuum packaging (VP) as a percentage of cold carcass weight of the respective animal

7.2 Addendum B

Table B Statistical significance of the effects of ageing method, time and sex on the fatty acid content (mg/g of muscle) of springbok *Longissimus thoracis et lumborum* muscle.

Fatty Acid	Method	Time	Sex	M*T	M*S	T*S
Myristic acid (C14:0)	0.382	0.876	**0.024**	0.586	0.515	0.526
Pentadecanoic acid (C15:0)	0.983	0.697	0.382	0.862	0.605	0.742
Palmitic acid (C16:0)	0.887	0.767	**0.002**	0.265	0.786	0.202
Palmitoleic acid (C16:1)	0.719	0.621	0.055	0.959	0.999	0.511
Stearic acid (C18:0)	0.803	0.887	**0.017**	0.084	0.935	0.242
Oleic acid (C18:1n9c)	0.914	0.484	**0.016**	0.957	0.794	0.170
Linoleic acid (C18:2n-6c)	0.557	0.801	0.299	0.517	0.058	0.225
γ-Linolenic acid (C18:3n-6)	0.606	0.675	0.366	0.640	0.474	0.145
α-Linolenic acid (C18:3n-3)	0.674	0.987	0.841	0.662	0.500	0.215
Arachidic acid (C20:0)	0.669	0.220	0.809	0.210	0.340	0.682
Gondoic acid (C20:1)	0.489	0.506	0.881	0.487	0.255	0.142
Eicosadienoic acid (C20:2n-6)	0.424	0.162	0.851	0.652	0.067	**0.023**
Dihomo-γ-linolenic acid (C20:3n-6)	0.487	0.988	0.591	0.080	0.192	0.918
Arachidonic acid (C20:4n-6)	0.591	**0.003**	0.051	0.586	0.587	0.053
Eicosatrienoic acid (C20:3n-3)	0.865	0.894	0.027	0.069	**0.006**	0.164
Eicosapentaenoic acid (C20:5n-3)	0.779	0.346	0.430	0.340	0.150	0.841
Σ SFA	0.797	0.921	**0.004**	0.170	0.911	0.245
Σ MUFA	0.932	0.492	**0.016**	0.951	0.790	0.179
Σ PUFA	0.639	0.956	0.264	0.194	**0.041**	0.210
PUFA:SFA ratio	0.933	0.752	**0.007**	0.076	0.066	0.315
Σ n-6 PUFA	0.506	0.898	0.449	0.442	0.102	0.196
Σ n-3 PUFA	0.799	0.806	0.167	0.093	**0.022**	0.263
n-6:n-3 PUFA ratio	0.442	0.315	0.883	0.685	0.856	0.576
Total fatty acid content	0.966	0.783	**0.004**	0.540	0.575	0.068

Σ SFA = C14:0 + C15:0 + C16:0 + C18:0 + C20:0

Σ MUFA = C16:1 + C18:1n9c + C20:1

Σ PUFA = C18:2n-6c + C18:3n-6 + C18:3n-3 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:3n-3 + C20:5n-3

Σ n-6 = C18:2n-6c + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6

Σ n-3 = C18:3n-3 + C20:3n-3 + C20:5n-3

Table B Effect of ageing method, time and gender on the fatty acid composition (mg/g of muscle) of springbok *Longissimus thoracis et lumborum* muscle (mean ± standard deviation).

Fatty acids	Ageing method		Ageing time (days)				Sex	
	Skin-on	VAC	6	7	8	9	Male	Female
Myristic acid (C14:0)	0.38 ± 0.23	0.46 ± 0.35	0.37 ± 0.31	0.46 ± 0.30	0.46 ± 0.25	0.41 ± 0.36	0.26^{b} ± 0.19	0.58^{a} ± 0.29
Pentadecanoic acid (C15:0)	0.15 ± 0.07	0.12 ± 0.09	0.14 ± 0.03	0.12 ± 0.07	0.19 ± 0.05	0.10 ± 0.12	0.12 ± 0.08	0.16 ± 0.07
Palmitic acid (C16:0)	0.36 ± 0.25	0.45 ± 0.33	0.47 ± 0.35	0.55 ± 0.38	0.33 ± 0.21	0.29 ± 0.16	0.27^{b} ± 0.16	0.54^{a} ± 0.33
Palmitoleic acid (C16:1)	1.46 ± 0.88	1.81 ± 0.80	1.77 ± 1.09	1.98 ± 0.71	1.36 ± 0.70	1.43 ± 0.87	1.45 ± 0.86	1.82 ± 0.81
Stearic acid (C18:0)	5.60 ± 2.07	5.67 ± 2.38	5.46 ± 2.64	5.06 ± 2.05	6.06 ± 1.76	5.96 ± 2.60	4.64^{b} ± 2.04	6.63^{a} ± 1.90
Oleic acid (C18:1n9c)	3.54 ± 3.63	3.82 ± 4.21	4.41 ± 4.66	5.48 ± 4.37	2.91 ± 4.06	1.92 ± 1.41	1.67^{b} ± 1.07	5.69^{a} ± 4.59
Linoleic acid (C18:2n-6c)	1.95 ± 0.95	2.01 ± 0.87	2.02 ± 0.98	2.26 ± 0.81	1.83 ± 0.91	1.79 ± 1.02	2.18 ± 1.00	1.77 ± 0.77
γ-Linolenic acid (C18:3n-6)	0.26 ± 0.19	0.24 ± 0.22	0.20 ± 0.18	0.25 ± 0.17	0.26 ± 0.22	0.30 ± 0.25	0.23 ± 0.22	0.28 ± 0.18
α-Linolenic acid (C18:3n-3)	0.84 ± 0.63	0.84 ± 0.59	0.82 ± 0.58	0.99 ± 0.49	0.76 ± 0.62	0.80 ± 0.80	0.84 ± 0.62	0.85 ± 0.60
Arachidic acid (C20:0)	0.34 ± 0.15	0.33 ± 0.13	0.28 ± 0.10	0.25 ± 0.10	0.40 ± 0.16	0.41 ± 0.11	0.34 ± 0.10	0.33 ± 0.17
Gondoic acid (C20:1)	0.10 ± 0.09	0.07 ± 0.04	0.07 ± 0.05	0.09 ± 0.05	0.08 ± 0.06	0.11 ± 0.11	0.09 ± 0.08	0.09 ± 0.06
Eicosadienoic acid (C20:2n-6)	0.04 ± 0.03	0.04 ± 0.04	0.04 ± 0.03	0.05 ± 0.04	0.04 ± 0.04	0.01 ± 0.03	0.04 ± 0.04	0.03 ± 0.03
Dihomo-γ-linolenic acid (C20:3n-6)	0.14 ± 0.11	0.11 ± 0.09	0.12 ± 0.08	0.14 ± 0.09	0.15 ± 0.11	0.09 ± 0.13	0.12 ± 0.10	0.14 ± 0.10
Arachidonic acid (C20:4n-6)	0.10 ± 0.09	0.08 ± 0.08	0.15^{x} ± 0.05	0.10^{x} ± 0.07	0.11^{x} ± 0.01	0.00^{y} ± 0.00nd	0.12 ± 0.10	0.07 ± 0.06
Eicosatrienoic acid (C20:3n-3)	1.97 ± 0.74	2.23 ± 0.91	1.89 ± 0.78	2.35 ± 1.16	2.08 ± 0.80	2.08 ± 0.60	2.42^{x} ± 0.95	1.77^{y} ± 0.52
Eicosapentaenoic acid (C20:5n-3)	1.29 ± 0.45	1.23 ± 0.41	1.06 ± 0.36	1.13 ± 0.53	1.41 ± 0.45	1.45 ± 0.26	1.35 ± 0.41	1.17 ± 0.43
Σ SFA	12.90 ± 4.99	12.75 ± 5.62	12.26 ± 5.48	12.21 ± 5.23	14.02 ± 5.19	12.80 ± 6.12	9.76^{y} ± 4.17	15.89^{x} ± 4.32
Σ MUFA	4.00 ± 3.83	4.35 ± 4.52	4.92 ± 4.94	6.12 ± 4.73	3.33 ± 4.25	2.32 ± 1.54	2.03^{b} ± 1.18	6.32^{a} ± 4.88
Σ PUFA	6.60 ± 2.58	6.78 ± 2.36	6.29 ± 2.63	7.27 ± 2.80	6.65 ± 2.39	6.52 ± 2.43	7.29 ± 2.43	6.08 ± 1.84
PUFA:SFA	0.62 ± 0.41	0.69 ± 0.44	0.63 ± 0.44	0.70 ± 0.46	0.60 ± 0.43	0.68 ± 0.45	0.87^{x} ± 0.44	0.43^{y} ± 0.26
Σ n-6	2.49 ± 1.21	2.48 ± 1.03	2.54 ± 1.22	2.80 ± 1.13	2.40 ± 1.20	2.19 ± 1.07	2.68 ± 1.25	2.29 ± 0.94
Σ n-3	4.11 ± 1.44	4.30 ± 1.42	3.76 ± 1.42	4.47 ± 1.79	4.25 ± 1.26	4.33 ± 1.39	4.61 ± 1.68	3.79 ± 0.97
n-6:n-3	0.59 ± 0.17	0.57 ± 0.15	0.65 ± 0.14	0.65 ± 0.14	0.54 ± 0.18	0.49 ± 0.12	0.57 ± 0.16	0.59 ± 0.15
Total fatty acid content	23.49 ± 8.02	23.88 ± 8.00	23.49 ± 10.30	25.61 ± 10.59	23.99 ± 6.52	21.65 ± 3.52	19.08^{y} ± 3.83	28.29^{x} ± 8.21

Table B continued

[a,b] means in the same row (within the main effect) with different superscripts differ significantly from each other $p \leq 0.05$

[x,y] means in the same row (within the main effect) with different superscripts differ significantly from each other $p \leq 0.01$

[nd] not detected

***** Percentage fatty acid calculated as the percentage of an individual fatty acid in a sample (µg/g of meat) to the total fatty acids recorded in the sample (µg/g meat)

Σ SFA = C14:0 + C15:0 + C16:0 + C18:0 + C20:0

Σ MUFA = C16:1 + C18:1n9c + C20:1

Σ PUFA = C18:2n-6c + C18:3n-6 + C18:3n-3 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:3n-3 + C20:5n-3

Σ n-6 = C18:2n-6c + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6

Σ n-3 = C18:3n-3 + C20:3n-3 + C20:5n-3

7.3 Addendum C

Table C The mean retention indices* of volatile compounds detected in springbok *Longissimus thoracis et lumborum* (LTL) muscle.

Volatile compound	Retention index
Decane	1000
Toluene	1021
Hexanal	1070
Methyl valerate	1076
3-pentanol	1110
2-pentanol	1120
Methyl 4-methyl pentanoate	1147
1-butanol	1135
1-penten-3-ol	1145
Methyl hexanoate	1165
D-limonene	1172
Furan 2-pentyl	1199
Ethyl hexanoate	1202
1-pentanol	1219
Methyl 2-ethyl hexanoate	1233
Acetoin (3-hydroxybutanone)	1245
Methyl heptanoate	1258
Methyl 2-hydroxypropanoate	1279
1-hexanol	1317
Methyl octanoate	1359
1-octen-3-ol	1412
1-heptanol	1416
1-hexanol-2-ethyl	1452
Methyl nonanoate	1461
1-octanol	1517
3,5-octadien-2-one	1509
2,3-butanediol	1543
Methyl decanoate	1564
Butyrolactone	1568
2-octen-1-ol	1572
Unidentified alcohol	1597
2,4- decadienal	1763
Methyl dodecanoate	1770
Hexanoic acid	1797
Benzyl alcohol	1807
Dimethyl sulphone	1815
Hexanoic acid, 2-ethyl	1861
Methyl tetradecanoate	–
Octanoic acid	–
Caprolactam	–
Methyl hexadecanoate	–

* $Retention\ index = 100 \times \left[\frac{t_x - t_n}{t_{n+1} - t_n} + n\right]$

t_x is the retention time of the compound of interest.

t_n is the retention time of the alkane eluting before the compound of interest.

t_{n+1} is the retention time of the alkane eluting after the compound of interest.

n is the carbon number of the alkane eluting before the compound of interest.

– Insufficient data from alkane mix to calculate these retention indices.